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# CONTENTS

1914

## No. 1 JANUARY

J. PARSONS SCHAEFFER AND LOUIS H. NACHAMOFKY. Some observations on the anatomy of the upper extremities of an infant with complete bilateral absence of the radius. Six figures.....	1
JAMES F. COBEY. An anomalous right subclavian artery. Two figures.....	15
J. DOUGLAS PERKINS, JR. An anomalous muscle of the leg: Peroneo-calcaneus internus. Three figures.....	21
R. M. STRONG. Some ideas in laboratory equipment. Four figures.....	27

## No. 2 FEBRUARY

CHARLES R. STOCKARD. The artificial production of eye abnormalities in the chick embryo. Two plates.....	33
R. A. MCGARRY. A case of patency of the pericardium and its embryological significance. One figure.....	43
GEORGE G. SCOTT. The percentage of water in the brain of the smooth dog-fish, <i>Mustelus canis</i> .....	55
RANDOLPH WEST. A note on the presence of a musculus cleido-atlanticus in the domestic cat ( <i>Felis domestica</i> ). One figure.....	65
Proceedings of the American Association of Anatomists. Thirtieth session: Abstracts: Demonstrations.....	69

## No. 3 MARCH

ARTHUR WILLIAM MEYER. The occurrence of supernumerary spleens in dogs and cats, with observations on corpora libera abdominalis. IV. Studies on hemal nodes. Twelve figures.....	147
J. PLAYFAIR McMURRICH. The nomenclature of the carpal bones.....	173

## No. 4 APRIL

J. B. JOHNSTON. The nervus terminalis in man and mammals. Nine figures.....	185
SAMUEL T. ORTON. A note on the circulation of the cornu ammonis. Two figures.....	199
ADAM M. MILLER AND J. E. MCWHORTER. Experiments on the development of blood vessels in the area pellucida and embryonic body of the chick. Thirteen figures...	203
Reproduction of models by The Wistar Institute.....	228
List of members of the American Association of Anatomists.....	229

## No. 5 MAY

J. G. KRAMER and T. W. TODD. The distribution of the nerves to the arteries of the arm, with a discussion of the clinical values of results. Five figures.....	243
HARRIS E. SANTEE. The brain of a black monkey ( <i>Macacus maurus</i> ): The relative prominence of different gyri. Four figures.....	257
CHARLES E. JOHNSON. An additional case of pancreatic bladder in the domestic cat. One figure.....	267
HAROLD L. KEARNEY. On the relative growth of the organs and parts of the embryonic and young dogfish ( <i>Mustelus canis</i> ).....	271
ROBERT BENNETT BEAN. The eruption and decay of the permanent teeth .....	299
ARTHUR WILLIAM MEYER. Osteology redivivus: A criticism.....	303

## No. 6 JUNE

BARTON G. DUPRE and T. WINGATE TODD. A transitional type of cervical rib, with a commentary. Four figures.....	313
GEORGE P. LEONHART. A case of stylo-hyoid ossification. Two figures.....	325
RICHARD W. HARVEY. A case of multiple renal arteries. One figure.....	333
J. R. DRIVER and A. B. DENISON. The morphology of the long accessorius muscle. Four figures.....	341
FRANKLIN PARADISE JOHNSON. A case of atresia ani in a human embryo of 26 mm. One figure.....	349
F. E. CHIDESTER. Cyclopia in mammals. Twelve figures.....	355
F. E. CHIDESTER. Twins in fish, one with a cyclopic deformity. Four figures.....	367

## No. 7 JULY

E. R. HOSKINS. On the vascularization of the spinal cord of the pig. Five figures ...	371
EDWARD F. MALONE. A course of correlational anatomy.....	393
FRANKLIN P. REAGAN. A useful modification of Mann's methyl-blue-eosin stain.....	401

## No. 8 AUGUST

ROSALIND WULZEN. The morphology and histology of a certain structure connected with the pars intermedia of the pituitary body of the ox. Seventeen figures.....	403
J. C. MILLER. Ossiculum lus.....	415
E. R. HOSKINS. Persistent arteriæ branchii superficialis, antibranchii superficialis et mediana. One figure.....	421

## No. 9 SEPTEMBER

H. E. JORDAN. The microscopic structure of mammalian cardiac muscle with special reference to so-called 'muscle cells.' Eight figures.....	423
R. R. BENSLEY. The thyroid gland of the opossum. Three figures.....	431
T. WINGATE TODD. Covers for dissecting tables. Three figures.....	441
T. WINGATE TODD. A tank for the preservation of anatomical material. Three figures	444
A. O. WEESE. A simple electrical heating device for incubators, etc. Four figures....	447

# CONTENTS

v

## No. 10 OCTOBER

VICTOR J. HAYS. The development of the adrenal glands of birds. Eight figures...	451
PAUL S. McKIBBEN. Mast cells in the meninges of <i>Necturus</i> and their differentiation from nerve cells. Two figures.....	475

## No. 11 NOVEMBER

ROBERT BENNETT BEAN. A racial peculiarity in the pole of the temporal lobe of the negro brain. Nineteen figures (three plates).....	479
LESLIE B. AREY. An abnormality in the intestine of <i>Necturus maculosus</i> Raf. Six figures.....	493
P. E. SMITH. The development of the hypophysis of <i>Amia calva</i> . Ten figures.....	499
RICHARD W. HARVEY. A brain macrotome. Two figures.....	507

## No. 12 DECEMBER

SHINKISHI HATAI. On the weight of some of the ductless glands of the Norway and of the albino rat according to sex and variety. Five charts.....	511
OTTO C. GLASER. On the mechanism of morphological differentiation in the nervous system. Three figures.....	525





## SOME OBSERVATIONS ON THE ANATOMY OF THE UPPER EXTREMITIES OF AN INFANT WITH COM- plete BILATERAL ABSENCE OF THE RADIUS

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*The Anatomical Laboratory of the Yale Medical School*

### SIX FIGURES

On February 20, 1913, the body of a white, male infant, aged one day, of average size and weight reached the anatomical rooms of the Yale Medical School. On inspection the body appeared to be well developed and normal, save for an odd-appearing deformity of both upper extremities. At autopsy the pleural cavities were found to contain a considerable amount of blood, doubtless the result of a birth injury.

Upon examination of the deformed upper extremities, it was found impossible to pronate or supinate the antebrachium and hand, save for a slight alteration in position allowed by turning the humerus and ulna on their vertical axes. The hands were fixed in marked adduction; the right one making a right angle and the left one considerably less than a right angle with the antebrachium. Both hands were rotated on the ulnae so that their dorsa presented ventrad, that is, they were fixed in pronation and abutted the ulnae at their medial aspects (figs. 1 and 5).

The position of the hands indicated lack of radial support. Careful examination failed to reveal any trace of a radius in either arm. A provisional diagnosis of complete absence of both radii was made. This was later confirmed by Röntgen rays and by dissection (figs. 2 and 5).

The ulnae seemed normal in position and size. However, due to the faulty position of the hands, the distal extremities of the ulnae formed very prominent subcutaneous points at the wrists (figs. 1 and 2).

The fact of observing the absence of both radii and of the resultant faulty position of the hands seemed of little value. It was, therefore, deemed advisable to make a careful dissection of at least one of the extremities to ascertain to what extent other anatomical errors were present. In order to study the feasibility of tendon transplantation in such cases, in an attempt to lessen the deformity and to increase the efficiency of the member, a dissection of the antebrachium and hand was deemed of especial value.

The dissection was done by the junior author (Nachamofsky, Class of 1916, Yale Medical School). Some of the facts disclosed by this dissection will be discussed in subsequent paragraphs.

#### A. MYOLOGY

One of the first important facts brought out by the dissection was the marked failure in the differentiation of many muscle masses into individual muscles. This led to only a partial differentiation of some muscles and to a complete absence of others. Throughout the upper extremity there was a marked failure in very many instances in the formation of tendons; the muscles arising or inserting by fleshy contacts where normally tendons are present.

Another fact to be noted is that those muscles normally associated with the radius, but in this case only partly differentiated, were found to attach to the ventral surface and lateral border of the ulna.

Not only the muscles of the antebrachium and hand but many of the brachium and shoulder likewise were found to be abnormal.

*Muscles of shoulder.* The origin of the deltoid muscle was approximately normal. Its insertion was, however, markedly altered. It passed distally from its origin; its fibers converging towards the lateral intermuscular septum, to which it gained insertion just proximal to the lateral epicondyle of the humerus.

The deltoid had no insertion on the humerus, but became continuous with the teres major dorsally; with the brachio-



Fig. 1. Sketch of an infant with complete bilateral absence of the radius. Especially note the abnormal position of the hands. The prominence at the wrist is caused by the distal extremity of the ulna. See text for further description of the upper extremities.



Fig. 2. Skiagraphs of the upper extremities of infant sketched in fig. 1. Note the characteristic position of the hands and the complete absence of both radii. As usual the carpal bones show no ossification at this age.

radialis and the extensor carpi radialis longus and brevis muscles distally; and more or less with the pectoralis major muscle ventrally.

The supraspinatus and the infraspinatus and the teres minor muscles were normal save that the latter and last were inseparably mingled.



The teres major muscle at its origin was undifferentiated from an abnormally extensive origin of the long head of the triceps brachii muscle. Contrary to the normal course of the muscle, in passing from origin to insertion, it coursed lateral to the upper extremity of the humerus and became continuous with the deltoid, the brachio-radialis, and the extensor carpi radialis longus and brevis muscles. At no point was the teres major muscle directly attached to the humerus.

The latissimus dorsi muscle was inserted by two distinct tendinous slips. Near its insertion the lateral and somewhat larger slip terminated in a fleshy band which became incorporated with the common mass of the heads of the triceps brachii muscle. The faulty course of this portion of the latissimus dorsi muscle, as well as that of the teres major muscle mentioned above should here be noted. A shorter medial head of the latissimus dorsi muscle coursed cephalically and ventrally, giving a tendinous portion to insert on the humerus just distal to the subscapularis muscle and a caudal fascial expansion which gave origin to a part of the medial head of the triceps brachii muscle.

The pectoralis major muscle in addition to its normal insertion sent fibers into the deep aspect of the deltoid muscle, thus forming an accessory muscular band one cm. wide.

*Muscles of brachium.* The biceps brachii muscle attempted an origin from the supraglenoid tubercle, and some of its tendinous fibers could be traced to it, but its long head mainly arose from the capsule of the shoulder joint which it materially strengthened. The short head of the muscle arose as usual from the coracoid process of the scapula. The belly of the biceps brachii inserted (?) along the distal half of the medial and lateral surfaces and the medial and lateral epicondylar ridges of the humerus. The interval between the epicondylar ridges was bridged over by some biceps brachii fibers which passed distally to insert onto the coronoid process of the ulna and to give origin to the extensor digitorum communis and the extensor digiti quinti proprius muscles. The canal thus formed between the epicondyles of the humerus transmitted the median nerve *laterally* and the brachial artery *medially*.

The brachialis (antecus) muscle as such was absent. The apparent absence of this muscle together with the fact that some biceps brachii fibers inserted on the coronoid process of the ulna leads one to believe that the brachialis was incorporated with the biceps brachii fibers and that it had not differentiated from it.

The coraco-brachialis muscle arose with the short head of the biceps brachii muscle from the coracoid process. It had an abnormally extensive insertion on the humerus and into the brachial fascia.

An acromio-humeral muscle appeared as an anomalous band, arising from the inferior surface of the overhanging acromion process and the capsule of the shoulder joint. It inserted on the humerus just lateral to the major tubercular crista. It lay beneath the deltoid muscle. This may explain the absence of a bony insertion of the latter muscle and might be considered an isolated deep portion of it.

The long head of the triceps brachii muscle arose very extensively not only from the infraglenoid tubercle but from a goodly portion of the axillary border of the scapula where it was intimately blended with the teres major muscle as mentioned in the previous paragraph. The medial head of the triceps brachii arose along the distal third of the dorsal surface of the humerus. The lateral head was partly incorporated with the long head and in part arose from the tendon of insertion of the latissimus dorsi muscle. As usual it gained insertion on the olecranon process of the ulna and into the antebrachial fascia in the immediate neighborhood.

The anconaeus muscle was normal in its origin but its area of insertion was abnormally extensive; the whole proximal half of the dorsal or extensor surface of the ulna was occupied by it.

*Muscles of the antebrachium and hand.* The brachio-radialis muscle took its origin from the distal fibers of the deltoid muscle and from the over-lying fascia. It inserted into the transverse carpal ligament and into the antebrachial fascia. The peculiar insertion of this muscle was probably due to the rotation of the hand about the ulna. The muscle took a rather devious course:

Turning ventrally from its origin, it occupied a position between the extensor carpi radialis longus and brevis muscles medially, and the extensor pollicis longus muscle (?) laterally, lying over a mass of undifferentiated muscular tissue.

The extensor carpi radialis longus and brevis muscles arose by a common fleshy head of origin from the caudal portions of the deltoid and the triceps brachii muscles. They coursed distally

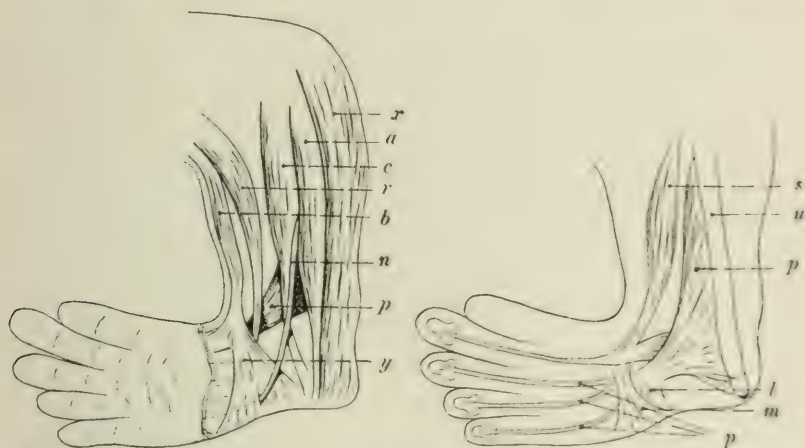


Fig. 3. Diagrammatic sketches of superficial (to the left) and deep (to the right) dissections of the ventral aspect of the right antebrachium and hand. The details of the dissection are purposely omitted. See text for description of figure. *x* = flexor carpi ulnaris muscle; *a* = palmaris longus muscle; *c* = flexor carpi radialis muscle; *r* = extensor carpi radialis longus et brevis muscles; *b* = brachioradialis muscle; *n* = ulnar nerve; *p* = flexor profundus digitorum muscle; *y* = hypothenar muscle; *s* = flexor digitorum sublimis muscle; *u* = ulna; *l* = lumbrical muscles undifferentiated; *m* = undifferentiated muscle.

over the latero-central aspect of the antebrachium, following the course of the brachio-radialis muscle. Both muscles inserted on the transverse carpal ligament medial to the brachio-radialis. The brevis was distinguishable from the longus only after they had traversed half their course (fig.3).

An extensor muscle of the thumb was present but did not correspond to any of the normal thumb extensors. It arose partly



from the most distal biceps brachii fibers and was intimately associated with a common muscle mass adherent to the ventral surface of the ulna. The muscle passed distally and became superficial at the region of the carpus where it was lateral to the extensor digitorum communis muscle. Its tendon gained the dorsum of the hand by passing through the first osteo-fibrous canal on the dorsum of the wrist and it inserted on the base of the distal phalanx of the thumb. On the dorsum of the hand a small muscular sheet arose from the thumb extensor which passed medially under the tendons of the other extensors to insert on the hypothenar fascia over the fifth metacarpal bone (fig. 4).

The extensor digitorum communis muscle was a round muscle which arose in common with the extensor digiti quinti proprius muscle from the distal fibers of the biceps brachii muscle, from the lateral epicondyle of the humerus, and from the antebrachial fascia. It passed superficially and distally through the second osteo-fibrous canal onto the dorsum of the hand. It coursed between the extensor digiti quinti proprius muscle medially and the extensor of the thumb laterally, lying over the ulna and an undifferentiated mass of muscle deeply placed. On the dorsum of the hand the extensor digitorum communis muscle gave off four tendons which inserted by broad fascial expansions on the phalanges of the second, third, fourth and fifth fingers. The more direct tendinous insertions were, however, to the distal segments (fig. 4).

The extensor digiti quinti proprius muscle arose in common with the extensor digitorum communis muscle. It was quite superficial on the antebrachium with the extensor carpi ulnaris muscle medial to it. On the dorsum of the hand its tendon divided, one part going to insert with a tendon from the extensor communis digitorum muscle and the other becoming continuous with the fascia over the fifth metacarpal bone (fig. 4).

The extensor carpi ulnaris muscle was normal except for the absence of a distinct tendon of insertion. The muscle terminated in a broad fascial band which inserted on the medial aspect of the distal extremity of the ulna. Its usual insertion on the fifth metacarpal bone was wanting.



The remaining muscles of the extensor group were either totally missing, as in the case of the supinator (brevis) muscle, or were not differentiated, but remained merely a muscle mass which lay between the extensor of the thumb and the flexors of the antebrachium. Since the flexor pollicis longus muscle was absent, it is likely that it had not differentiated from this mass. It should here be noted that the absent and undifferentiated muscles in the specimen are normally intimately associated with the radius, both with respect to their origin and their course.

The flexor carpi ulnaris muscle was normal in size and position. It, however, lacked a clean-cut tendon and did not find an insertion on the carpus. The only point of insertion was to the capsule of the joint between the ulna and the carpus.

The palmaris longus muscle (?) arose together with the flexor carpi ulnaris from the medial epicondyle of the humerus, and passed into the antebrachium lateral to the latter muscle. The palmaris longus and the flexor carpi ulnaris had a common insertion on the capsule of the joint between the carpus and the ulna (fig. 3).

The flexor carpi radialis muscle (?) took its origin from the medial epicondyle of the humerus in common with other flexors, and from the distal fibers of the biceps brachii and the intermuscular septum. It passed lateral to the palmaris longus (?) and was separated from it in the distal half of the antebrachium by the ulnar nerve. The fibers of the muscle converged to a point at the junction of the middle and distal thirds of the antebrachium. From this point the muscle again spread out into a triangular muscular sheet to ultimately insert on the ventral surface of the ulna and the proximal aspect of the carpus. It is probable that the flexor carpi radialis muscle in its distal third is normally more or less supported and directed by the radius. The latter being absent, the muscle dropped to a secondary support on the ulna (fig. 3).

The pronator (radii) teres muscle was entirely wanting.

The flexor digitorum sublimis muscle (?) arose by two heads: that from the medial epicondyle was extremely small, and barely extended to this bony point. It also gained a slight origin

from the medial intermuscular septum. The radial head of the muscle dropped more distally and deeply, due to the absence of the radius, and arose from the lateral border of the ulna near the carpus. The latter origin was found on a deeper level than that of the flexor digitorum profundus muscle, and in its passage into the hand it lay ventral to the mass of thenar muscles. The two heads joined in the hand to form a distinct tendon which inserted on the base of the distal phalanx of the index finger. In the palm of the hand a sheet of muscle tissue extended from the tendon of the flexor sublimis digitorum (?) over the flexor profundus digitorum towards the fifth metacarpal bone. It was not clear what this muscle represented (fig. 3).

The flexor digitorum profundus muscle arose beneath the superficial muscles from the whole ventral surface of the ulna. It lay ventral to the second head of the flexor digitorum sublimis muscle. The muscle was fan-shaped, converging to a point on the carpus and continuing into a tendon which sent three slips to the bases of the distal phalanges of the third, fourth and fifth fingers (fig. 3). In the palm the tendon passed beneath the flexor sublimis digitorum. From the ventral surface of the tendon an undifferentiated sheet of muscle extended toward the fifth metacarpal bone and lay beneath the accessory sheet of muscle given off from the flexor digitorum sublimis. From this muscular mass two lumbrical muscles were given off for the fourth and fifth fingers (fig. 3).

The thenar muscles were represented by a small mass of undifferentiated muscular tissue. This muscular mass arose from the superficial fascia and from the undifferentiated extensor muscular mass. It extended to the base of the second phalanx of the thumb. A distinct tendon for the mass was wanting.

The extensor pollicis longus muscle was absent as a distinct muscle. It was probably incorporated in the undifferentiated extensor mass.

The hypothenar muscles were not differentiated into individual muscles, but together formed a triangular sheet of muscle which arose from the transverse carpal ligament. The muscular sheet inserted on the medial border of the fifth metacarpal bone.

The pronator quadratus muscle was probably represented by a mass of muscular tissue which surrounded the distal extremity of the ulna (fig. 4).

The palmar interossei muscles were normal.

The dorsal interossei muscles were absent except the one for the index finger.

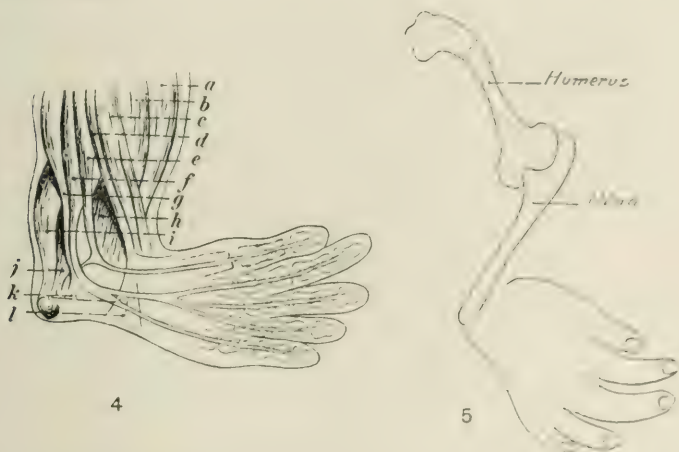


Fig. 4. Diagrammatic sketch of a dissection of the lateral aspect of the antebrachium and of the dorsum of the hand of the right upper extremity. Only the muscles are indicated. *a* = flexor carpi ulnaris; *b* = palmaris longus and flexor carpi radialis (?) muscles; *c* = extensor carpi radialis longus et brevis muscles; *d* = brachio-radialis muscle; *e* = extensor of thumb; *f* = extensor digitorum communis muscle; *g* = extensor digiti quinti proprius muscle; *h* = undifferentiated muscle; *i* = extensor carpi ulnaris muscle; *j* = pronator quadratus muscle (?); *k* = tendons of extensor digiti quinti proprius muscle; *l* = sheet of muscle from thumb extensor.

Fig. 5. Outline drawing of the bones of the right brachium and antebrachium after the muscles were removed. The hand is also shown in its fixed position.

## B. OSTEOLOGY

The humerus was shorter than normal, measuring only 5 cm in length. The proximal epiphysis was relatively extensive, 1.9 cm. in length. It projected cephalically and ventrally from the shaft at an angle of about  $135^{\circ}$  (fig. 5). The shaft of the humerus was more or less rounded and not easily divisible into surfaces and borders.



The capsule of the elbow joint was very lax and thin. It had incorporated in it many muscle fibers, and the differentiation of the various ligaments of the joint was very slight. The size of the capsular ligament permitted a rather complex elbow motion. Not only was the normal ginglymoid movement possible in its full extent, but a distinct trochoidal movement of the ulna on the humerus was also possible. This condition in a measure compensated for the absence of the radius and made a small degree of pronation and supination of the antebrachium possible.

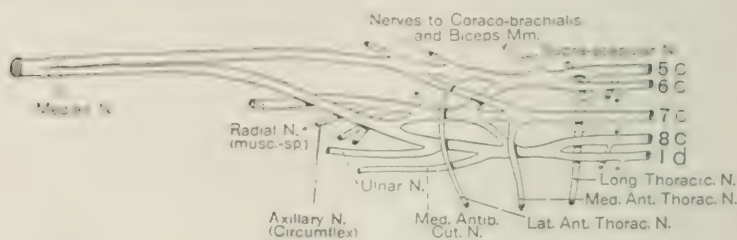


Fig. 6. Diagram of the brachial plexus of the right upper extremity. See text for description of it.

It should also here be noted that the medial aspect of the distal extremity of the ulna formed a diarthrodial joint with the carpus.

The osteology of the hand and carpus appeared normal for the age of the infant.

#### C. NEUROLOGY

The distribution of the nerves was more or less normal, but the altered musculature necessarily complicated the arrangement of the nerves. However, the nerve supply was an aid in differentiating the various muscles of the antebrachium and hand. The one striking thing about the nerves throughout the upper extremity was the unusually large size of the main trunks. The brachial plexus also deviated from its normal arrangement. As is indicated in the diagram of the plexus (fig. 6), the medial and lateral components of the median nerve remained independent to the level of the bend of the elbow. Here the components



united to form the median nerve proper. Another peculiar condition of the median nerve was the origin of its medial component (inner head) from both the medial and dorsal cords of the plexus. The musculo-cutaneous nerve (?) ended in the substance of the biceps muscle and another small nerve from the lateral cord ended in the coraco-brachialis muscle. In the diagram these nerves are designated "nerves to coraco-brachialis and biceps muscles."

The medial anterior thoracic nerve was a branch of the middle trunk. At least the nerve so designated filled the description of the medial anterior thoracic nerve in every way save its point of origin.

The medial brachial (lesser internal) cutaneous nerve was entirely absent.

#### D. CONCLUSIONS

The agenesis of the radius in this case must have been due either to a failure of the radial portion to give rise to an anlage, or if the latter were established, some affection must have destroyed the skeleton anlage after it had begun to differentiate. In view of the fact that there was a complete absence of the radius the natural inference is that there was a lack of origin of the element.

It is difficult to say to what extent the absence of the radius was responsible for the marked errors in the musculature of the upper extremity. Certainly the absence of radial support and stimulus must have to a great extent influenced the muscles that normally arise and insert on this bone (it will be recalled that the latter muscles were in many instances profoundly altered).

Other antebrachial muscles, as well as those of the hand showed marked errors. The faulty position of the hand, doubtless primarily caused by lack of radial support, may have been responsible for some muscle alterations, especially those of the hand and those that normally insert on the carpus. Lack of radial guidance and stimulus may have influenced others. It is, however, difficult to see how the absence of the radius could have had any bearing on the development of the muscles of the shoulder and proximal half of the brachium. Notwithstanding, many of these

muscles were quite anomalous in their anatomy, as is indicated in the text.

It would, therefore, seem that the whole error-complex of the upper extremity was primarily due to the lack of a proper formative stimulus or stimuli, and that the absence of the radius could merely account for secondary muscular errors due to the lack of support and stimulus normally supplied by this bone. The faulty position of the hand seemed purely secondary, due to lack of radial support and muscular contraction.

## AN ANOMALOUS RIGHT SUBCLAVIAN ARTERY

JAMES F. COBEY

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TWO FIGURES

Among the variations in the arrangement of the branches of the aortic arch in man, there is an unusual anomalous condition in which the right subclavian artery arises from the arch distal to the left subclavian. An example of the aforementioned anomaly was met with by the writer in the dissecting room of the Yale Medical School during the session of 1912-1913.

A description of the specimen, with an attempt to explain the embryology thereof and with a suggestion of symptoms that might arise therefrom, is offered here in brief.

### DESCRIPTION

The anomaly to be considered was noted in the dissection of a male, negro cadaver, aged approximately forty-five years. The right subclavian artery, instead of arising normally in conjunction with the right common carotid from the innominate artery, came off as an entirely separate artery from the descending limb of the aortic arch on the left side of the body after the left subclavian artery was given off (fig. 1). The anomalous vessel reached the right arm by passing dorsal to the trachea and the esophagus across the vertebral column.

The right subclavian took its origin from a point on the dorsal aspect of the descending limb of the aortic arch  $\frac{1}{2}$  inch distal and to the right of the normally placed left subclavian artery (fig. 1). Leaving the aorta, it passed at an angle of 45 degrees to the right and cephalad between the esophagus and the bony vertebral column. This direction was maintained in its further course through the neck with but a slight lateral curve to the point where the first branches arose from it.

As is indicated (fig. 1), the right vertebral artery was the first branch of the anomalous right subclavian and it came off at a distance of  $4\frac{1}{2}$  inches from the origin of its parent from the aorta. It therefore corresponded to the normal position for the vertebral artery—a fact worthy of note on account of differences in the position of the vertebral artery in cases of anomalous subclavian arteries like this. The right subclavian artery was crossed in the root of the neck by the right pneumogastric (vagus) nerve, but the inferior (recurrent) laryngeal branch of the vagus did not hook around the subclavian artery as usual,—that is, it was not recurrent, as shown in figure 1.

There was no innominate artery present in the subject, both common carotid arteries arising separately from the arch of the aorta. The relations of the second and third parts of the right subclavian artery and the origin and distribution of its branches were perfectly normal.

#### EMBRYOLOGIC EXPLANATION

Embryology readily explains the anomaly described above by assuming a defect in the absorption of the primitive right aortic arch in the embryo. Absorption occurs ordinarily distal to the point of origin of the right subclavian artery (*a* to *b*, fig. 2 *B*). In the case in question the position of absorption was along the fourth branchial arch (*a* to *c*, fig. 2 *C*). The embryonic right aorta became the right subclavian artery (*a* to *x*, fig. 2 *C*).

The position of the right subclavian so far cephalad on the left arch (arch proper) of the aorta may be explained in two ways: Either there was a positive migration of the artery from point *X* in figure 2 *C* to point *x* in figure 2 *D*, or the migration is only apparent and the real change was a dragging down of the aortic arch in the mid-line by a downward movement of the heart and pulmonary system. Probably both processes progressed *para passu* and had a share in bringing about adult conditions found in this case. The second view gives a reason for the position of the right subclavian artery dorsal to the trachea. The pulmonary system descends ventrally with the heart through



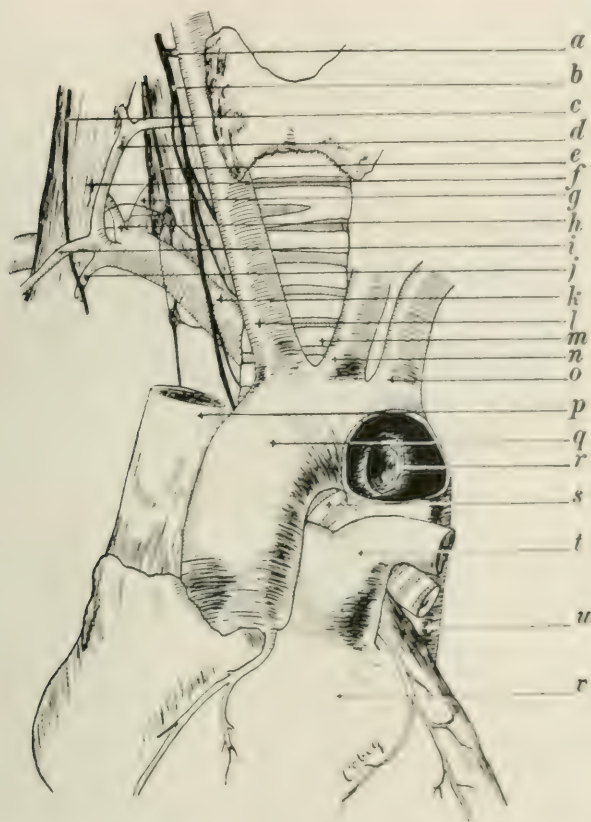


Fig. 1 Drawing from a dissection of the ventral aspect of the neck and thorax showing origin, course, and relations of the anomalous right subclavian artery. A window is cut into the aorta to show point of origin of anomalous vessel.

- |   |  |
|---|--|
| <i>a</i> , recurrent (inferior) laryngeal nerve | <i>m</i> , trachea                                     |
| <i>b</i> , vagus nerve                          | <i>n</i> , left common carotid artery                  |
| <i>c</i> , phrenic nerve                        | <i>o</i> , left subclavian artery                      |
| <i>d</i> , inferior thyroid artery              | <i>p</i> , superior vena cava                          |
| <i>e</i> , sympathetic cord                     | <i>q</i> , aorta                                       |
| <i>f</i> , anterior scalene muscle              | <i>r</i> , origin of anomalous right subclavian artery |
| <i>g</i> , vertebral artery                     | <i>s</i> , left bronchus                               |
| <i>h</i> , transverse cervical artery           | <i>t</i> , pulmonary artery                            |
| <i>i</i> , suprascapular artery                 | <i>u</i> , descending thoracic aorta                   |
| <i>j</i> , internal mammary artery              | <i>v</i> , heart                                       |
| <i>k</i> , anomalous right subclavian artery    |  |
| <i>l</i> , right common carotid artery          |  |

the heart-shaped space (figs. 2 *C* and 2 *D*) which is surrounded by the two embryonic aortae, so that the trachea comes to lie ventral to the anomalous subelavian artery when this is formed. The esophagus necessarily occupies the same position because, since the large blood vessels in the region develop around the fore-gut, the latter lies from the first in the loop ventral to the

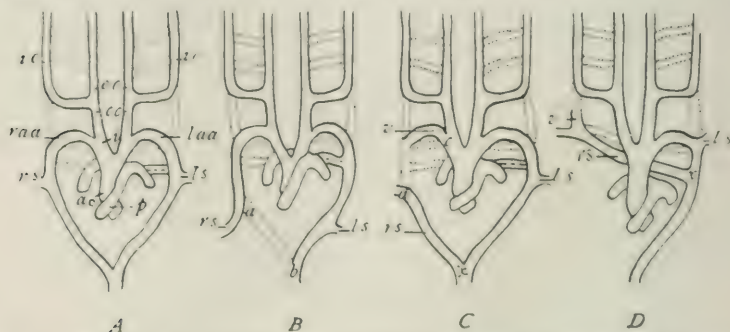


Fig. 2 Series of diagrams of the embryonic arterial arches for comparison of the normal with the abnormal development of the right subelavian artery. Dotted lines indicate points of absorption.

*A* Diagram representing primitive conditions.

*B* Diagram representing the usual development.

*C* Diagram showing the anomalous right subelavian artery (diagrams *A*, *B*, and *C* were modified from Piersol's Anatomy).

*D* An original diagram to illustrate subsequent change in position of anomalous right subelavian artery to condition described in text. The formation of the right vertebral artery should be noted also.

*ic*, internal carotid artery  
*ec*, external carotid artery  
*cc*, common carotid artery  
*raa* and *laa*, right and left aortic arches respectively  
*i*, innominate artery

*rs* and *ls*, right and left subelavian arteries respectively  
*ao*, aorta  
*p*, pulmonary artery  
*v*, vertebral artery  
*x*, point of origin of anomalous right subelavian artery

dorsal aortae, and will therefore be ventral to an anomalous right subelavian artery as found in this cadaver, since the artery represents in part the original right dorsal aortic arch.

The exact extent and position of the absorption would appear to determine the point of origin of the right vertebral artery. That is, if the absorption of the right arch were not complete

medially, as in figure 2 *C*, the unabsorbed portion would persist as the right vertebral artery which might thus arise from the aorta or even from the right common carotid artery. In my specimen the absorption was apparently complete, however, and the vertebral artery arose directly from the right subclavian (fig. 2 *D*).

The frequency with which such anomalies of the subclavian artery occur is represented in a report published by Arthur Thomson. This report is the result of the efforts of a committee of collective investigation for the Anatomical Society of Great Britain and Ireland. Five hundred cases in all were examined and, of these, five (1 per cent) presented the anomalous condition in which the right aortic arch persisted distally.

#### APPLICATION

It is within the range of possibility that the pressure exerted upon the right subclavian artery in its position dorsal to the trachea and esophagus, as reported above, might produce symptoms resembling those of cervical rib. The natural inference would be that owing to pressure on the artery there would result a strong pulsation of the vessel and maybe trophic changes in the arm, forearm, and hand. Other symptoms and conditions associated with pressure on large blood vessels might be expected.

Anatomically the right arm appeared perfectly normal and further dissection of the body showed no other anomalies.

I am indebted to Prof. J. Parsons Schaeffer for suggestions and for reading the manuscript.





## AN ANOMALOUS MUSCLE OF THE LEG: PERONÆO-CALCANEUS INTERNUS.<sup>1</sup>

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### THREE FIGURES

The cadaver presenting this anomaly was that of a muscular negro of unknown age, and was one being used for ordinary dissection purposes in the Laboratory of Anatomy of the University of Pennsylvania. The condition was found to be bilateral, the muscle being present in both the right and left legs.

It is a flat muscle arising partly by digitations from the flexor hallucis longus and partly from the lower half of the mesial surface of the fibula. Its fibers pass downward and inward into a tendon at its internal border. The tendon courses downward and forward under the ligamentum laciniatum and over the sustentaculum tali to be inserted into the periosteum, and also into a tubercle at the distal internal surface of the calcaneus, just superior and lateral to the tendon of the flexor hallucis longus. At its origin it overlies the flexor hallucis longus; lower down the tendon enters the same compartment with that of the flexor hallucis longus, occupying a position superior and lateral to the latter.

The nerve supply could not be worked out as it had been destroyed before the writer took up the dissection, but it probably came from the same muscular branch as that which supplied the flexor hallucis longus, that is, from the nervus tibialis.

The blood supply is effected by means of a branch of the arteria peronæa.

The action is to assist in extension of the carpus and very slightly to aid in supination.

<sup>1</sup> From the Laboratory of Anatomy of the University of Pennsylvania.

Associated with this muscle, there were found several other anomalies in the plantar region.

The tendon of the flexor hallucis longus broke up into two slips, the extra one later dividing into two, which went to the second and third digits. At the lateral side of the distal forking, the lumbrical of the fifth digit took origin.

The insertion of the quadratus plantae divided and surrounded the branch of the flexor digitorum longus to the fifth digit.

A very slender slip of tendon connected the tendons of the flexor digitorum brevis and the flexor digitorum longus of the fifth digit.

As far as the writer has been able to determine, this anomaly was first reported by Alexander Macalister,<sup>2</sup> who gave the muscle its name. He describes it as follows:

Peroneo-calcaneus internus is a small muscle which . . . . . seems to resemble the tensor of the synovial membrane of the ankle of Henle and Linhart, . . . . . it arises below the flexor hallucis longus from an oblique line on the back of the fibula behind the external malleolus, passes over the back of the sustentaculum tali, in the groove with the flexor hallucis to be inserted into a tubercle of the os calcis. This muscle, I have elsewhere referred to as the probable homotype of the pronator quadratus.

M. Auvray reports later, in 1896:<sup>3</sup>

*Muscle surnuméraire de la région profonde postérieure de la jambe.* Faisceau péronéo calcaéen interne. Macalister a décrit sous ce nom un 'petit faisceau' se détachant audessous du fléchisseur propre du gros orteil de la face postérieure du péroné et venant se terminer sur le tubercle du calcanéum. Je reporte un fait double de cette anomalie musculaire, rencontré sur le même sujet. Dans mon cas, il ne s'agit pas d'un petit 'faisceau' comme le dit l'auteur précédent, mais de deux véritables muscles nettement distincts des muscles voisins.

Sur la jambe gauche le muscle est représenté dans ses deux tiers supérieurs par un corps charnu situé au-dessous et en dehors du long fléchisseur propre du gros orteil. Les fibres viennent converger obli-

<sup>2</sup> Transactions of the Royal Irish Academy (1872); vol. 25, Science, part 1, p. 125. Additional observations on muscular anomalies in human anatomy, Alexander Macalister.

<sup>3</sup> Bulletins de la Société Anatomique de Paris, 71<sup>e</sup> Année, (1896), 5<sup>me</sup> Serie, Tome 10., F. 7, p. 223-224. Anomalies musculaires et nerveuses. M. Auvray.

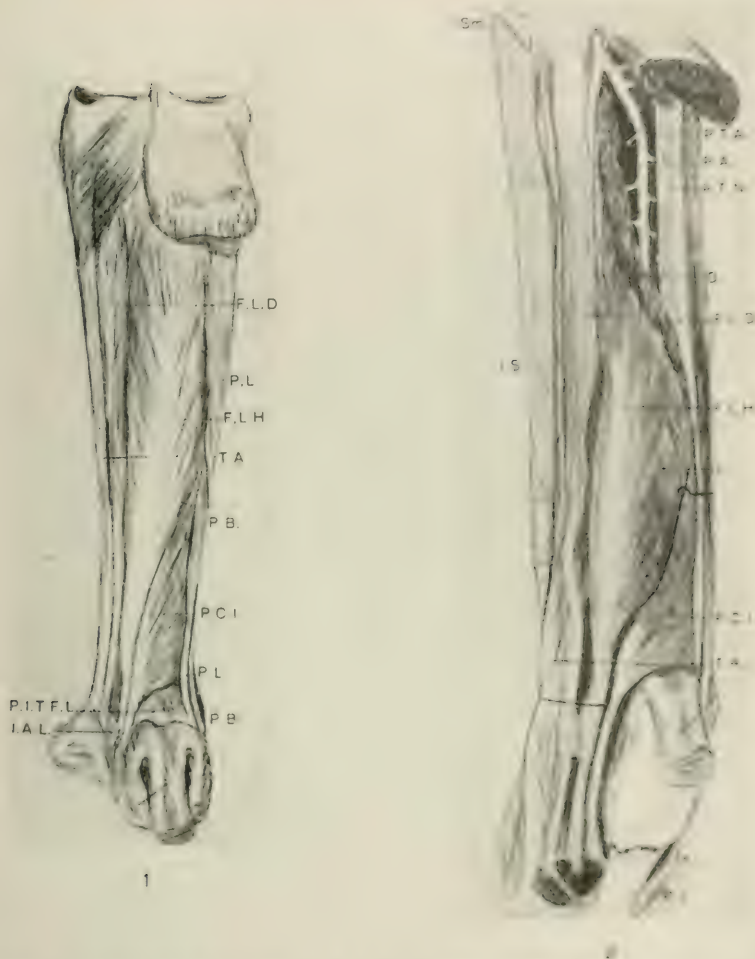


Fig. 1. Superficial dissection. *F.L.D.*, M. flexor digitorum longus; *P.L.*, M. peroneus longus; *F.L.H.*, M. flexor hallucis longus; *T.A.*, M. tibialis anticus; *P.B.*, M. peroneus brevis; *P.C.I.*, M. peroneo-calcaneus internus; *P.I.T.F.L.*, Lig. Malleoli lateralis posterior; *I.A.L.*, Lig. laciniatum.

Fig. 2 Deep dissection. *Sm.*, insertion of M. semimembranosus; *P.T.A.*, A. tibialis posterior; *P.A.*, A. peronea; *P.T.N.*, branch of N. tibialis; *O.*, origin of the M. flexor hallucis longus cut away from the fibula; *F.L.D.*, M. flexor digitorum longus; *F.L.H.*, M. flexor hallucis longus; *I.*, interdigitation between M. flexor hallucis longus and the M. peroneo-calcaneus internus; *P.C.I.*, M. peroneo-calcaneus internus; *T.A.*, M. tibialis anticus; *In.*, insertion of M. peroneo-calcaneus internus; *P.L.*, M. peroneus longus; *I.S.*, septum musculare posterius.

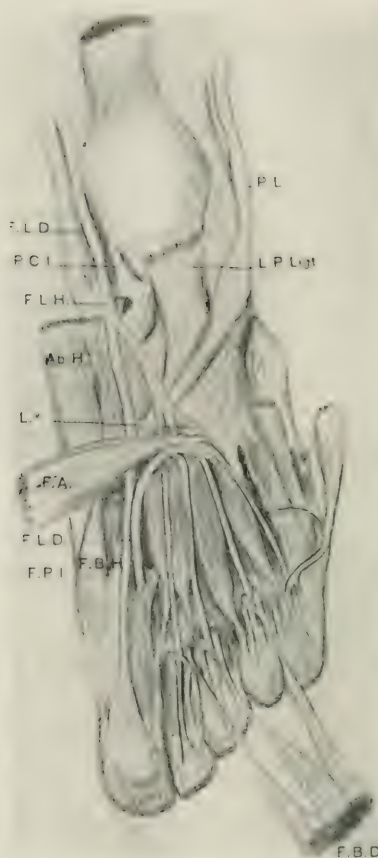


Fig. 3 Plantar region. *F.L.D.*, *M. flexor digitorum longus*; *P.C.I.*, *M. peronaeo-calcaneus internus*; *F.L.H.*, *M. flexor hallucis longus*; *Ab.H.*, *M. Abductor hallucis*; *L.*, *M. lumbricalis* of 5th digit; *F.A.*, *M. quadratus plantae*; *F.B.H.*, *M. flexor hallucis brevis*; *F.P.I.*, first plantar interosseus muscle; *P.L.*, *M. peronaeus longus*; *L.P. Lig't.*, long plantar ligament; *F.B.D.*, *M. flexor digitorum brevis*.

quement vers un tendon qui s'étend sur toute la longueur du muscle. Ce tendon passe sur la face interne du calcanéum dans la même gouttière que le long fléchisseur propre, et vient s'insérer dans le fond de la gouttière calcanéenne interne sous la chair carrée.

Sur la jambe droite, il s'agit d'un muscle qui est le plus volumineux des muscles de la couche profonde de ce côté. Il s'insère par son corps charnu sur la face postérieure du péroné dans toute son étendue, entre les in-



sertions des péroniers latéraux en dehors et du long fléchisseur propre en dedans. Les fibres charnues convergent obliquement vers un tendon qui occupe la face postérieure du muscle, s'en détache en arrière de la tibio-tarsienne pour passer dans la même coulisse tendineuse que le long fléchisseur propre, et s'insérer comme du côté opposé au fond de la gouttière calcanéenne sous la chair carrée.

A. F. Le Double<sup>1</sup> speaks of it as follows:

Par deux faisceaux fixés, l'un au tibia, l'autre à l'aponévrose qui recouvre le fléchisseur tibial et aboutissant à un tendon commun qui se divise, à la plante du pied, en deux branches dont la plus interne va se perdre sur le tendon du fléchisseur du gros orteil. Ces faisceaux ont été décrits sous le nom de *M. peroneo calcaneus internus* par M. Macalister qui les a découverts. M. Auvray en signale récemment un nouveau cas 1896.

The following mention is made of it by L. Testut:<sup>2</sup>

Faisceau péronéo-calcaneus interne (*Peroneo-calcaneus internus* de Macalister). C'est un petit faisceau, décrit par Macalister, se détachant, au-dessous du fléchisseur propre du gros orteil, de la face postérieure du péroné et venant se terminer sur le tubercule de calcaneum. Macalister, qui l'a décrit le premier, le rapproche du faisceau tenseur de la synoviale du cou-de-pied. Ne pourrait-on pas le rapprocher, avec autant de raison, du long accessoire des fléchisseurs.

The specimen was examined by Dr. George A. Piersol, who stated that he regarded the *peroneo-calcaneus internus* as an accessory long flexor.

With the idea in view that a prototype might exist among the mammalia, the writer made a search of the literature concerning the myology of extremities, and was unable to find mention of a similar muscle represented in any group.

The author wishes to express his gratitude to Dr. George A. Piersol, Professor of Anatomy, and to Dr. George Fetterolf, Assistant Professor of Anatomy, under whose direction the work has been done; also to J. Percy Moore, Ph.D., and Merkel H. Jacobs, Ph.D., for their assistance.

<sup>1</sup> *Traité des variations du système musculaire de l'homme* (1897). Tome 2, p. 404, Le Double.

<sup>2</sup> *Traité des variations du système musculaire de l'homme et de leur signification au point de vue de l'anthropologie zoologique*, Paris, 1897. L. Testut.

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## SOME IDEAS IN LABORATORY EQUIPMENT

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### FOUR FIGURES

In a previous paper<sup>1</sup> I described some electrical heating apparatus for paraffin baths in use at the University of Chicago. Recently, some improvements have been made, and I discuss these and some other laboratory apparatus, in this article.

In the paper just mentioned, I described a new form of thermostat designed for the Lillie type of paraffin bath. Though this thermostat runs for a number of weeks without attention, it occasionally becomes necessary to clean it, and one improvement consists in making the mounting more simple for the needle which makes and breaks the current to the 'cut out.' In the new form, the tube branch to be cleaned may be made accessible by removing only one screw which has a milled head as may be seen in figure 1. The tube branch is cleaned by a swab of cotton wrapped around the roughened end of a slender rod and dipped in nitric acid. In case the cotton slips off the rod and becomes packed in the tube, another rod with a barbed point is used to remove it.

Another improvement consists in omitting the flanges at the tops of the two branches of the glass tube. It was found that the preparation of these flanges often involves, even in the hands of a skilled workman, a slight contraction of the inside diameter of the tube branch at its top which prevents a perfect fitting for the adjusting-screw plug. If the latter does not completely close the tube branch at all points, mercury slips up above it and interferes seriously with the accuracy of the thermostat.

The mounting of a paraffin bath recently installed in the zoological laboratories of The University of Chicago is shown in figure 2. It is constructed of angle iron 39 mm. wide on the side; and it is 108 cm. high at the bottom of the paraffin bath. A trough for dripping paraffin will be noticed in the shelf. It increases in depth from 15 mm. at the left end to 30 mm. at the right, and it is 15 mm. wide. This paraffin bath was obtained from the Spencer Lens Company, and it is 59 cm. wide, 64 cm. high, and 39 cm. deep. It will be noticed that the cross section

<sup>1</sup> R. M. Strong. Electrical heating of paraffin baths. *Anat. Rec.*, vol. 7, no. 1, January, 1913, pp. 9-16; 6 text figures.



Fig. 1 From photograph of improved upper portion of thermostat

area of the mounting is a few inches wider and deeper than that of the bath, for the sake of stability. The sliding door was made as large as possible to furnish easy access to the electrical stove inside.

The positions of the thermostat and of the automatic switch, described in my previous paper, are indicated in figure 2. The switch box should ordinarily be at the opposite end of the bath from that shown in figure 2.



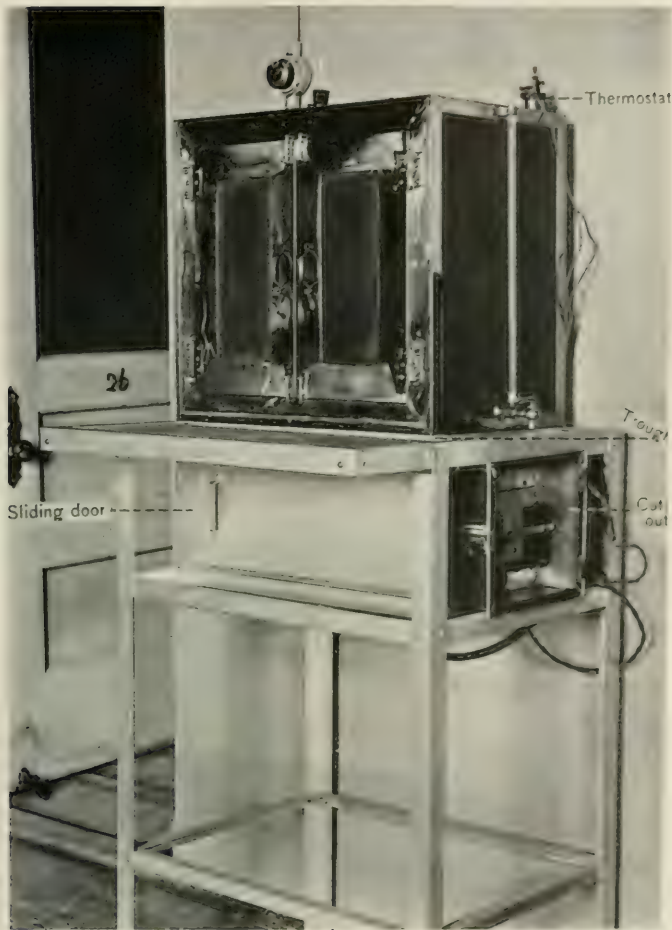
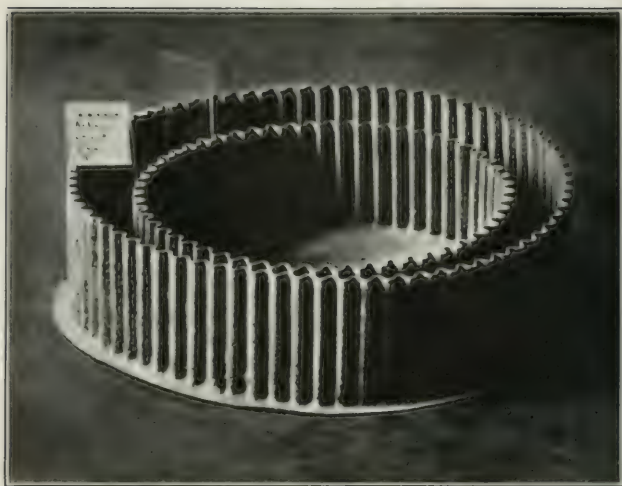
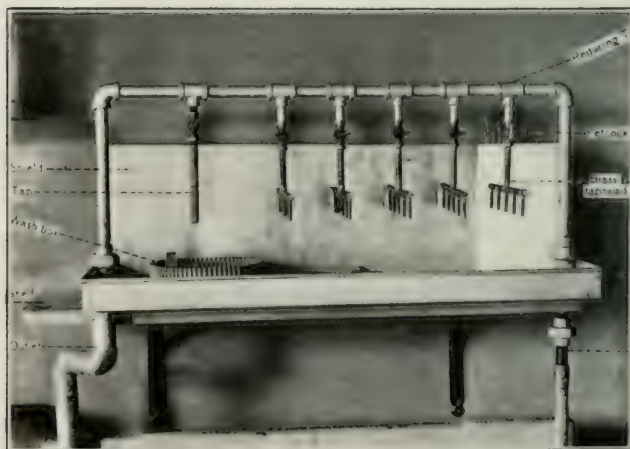


Fig. 2 From photograph showing mounting of paraffin bath with parts of electrical heating apparatus in view.

A box for washing microscope slides is shown in figure 3. While writing this paper, I was informed that similar boxes have been used elsewhere, but the idea may not be familiar to many workers. I have used this box with satisfaction for several years. It is constructed of corrugated, galvanized iron, and its dimensions are as follows: greater diameter  $7\frac{3}{4}$  inches, inner chamber  $5\frac{3}{4}$  inches. This leaves a space just wide enough for a 1 by 3 inch slide. The outside wall is two inches high, and that of the inner chamber is  $1\frac{3}{4}$  inches high. The box is placed



3



4

Fig. 3 From photograph of box for washing microscope slides.

Fig. 4 From photograph of apparatus for washing material in bottles or dishes and also for the box shown in figure 3.

under a tap as in figure 4, and water flows out over the top of the inner chamber into the slide chamber and from there out over the outer edge. A gentle circulation of water is thus provided in all parts of the slide chamber, which I tested by making the water densely turbid with a few drops of fuchsin. In the course of a very few minutes, all traces of the

stain disappeared from the slide chamber even with a rather gentle fall of water into the inner chamber. It is important to have the box in a horizontal position. It will be noticed in fig. 3 that labels at the upper end of the slide are not touched by the water.

Apparatus for washing histologically fixed tissues is shown in figure 4. This stands on a shelf above a sink, and the box is 82 cm. long, 5 cm. high, and 30 cm. wide. A series of small taps are provided, and all may be kept running by water at very moderate pressure, which enters at the right. Reducing T's lead to brass pet cocks and branched taps. At the left is seen the wash box which appears in figure 2. The pipe is plugged at the left of the tube which stands over the wash box. It is at once apparent that a number of bottles or jars of material may be washed simultaneously, an arrangement which is useful with classes in histology. An outlet of ample capacity, at the outer left hand corner, empties the box effectively.

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*(Continued)*

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DIE BIOLOGISCHEN GRUNDLAGEN DER SEKUNDÄREN GESCHLECHTSCHARAKTERE, von Dr. Julius Tandler, o.ö. Professor der Anatomie an der Wiener Universität, und Dr. Siegfried Grosz, Privatdozent für Dermatologie und Syphilidologie an der Wiener Universität. 23 text figures, 169 pages including index, 1913, 8 marks unbound and 8.80 marks bound. Verlag von Julius Springer, Berlin.

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## THE ARTIFICIAL PRODUCTION OF EYE ABNORMALITIES IN THE CHICK EMBRYO

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TWO PLATES

During the springs of 1911 and 1912 a series of experiments were conducted on hens' eggs aiming towards a definite modification of development so as to produce typical defects. A large number of eggs was used and numerous methods of treatment with various chemical stimuli were employed. The results, however, have not been of a definite nature, nevertheless they do indicate a decided tendency on the part of the developing central nervous system to respond to certain classes of stimuli in rather typical fashions. The responses are not in any sense specific for a given treatment but the same rather definite response may be obtained by a number of methods. This statement applies equally to other experiments on the artificial production of definite defects in the embryo. The earlier view that these defects were specific responses to the given chemical substance employed as has been advocated by Herbst,<sup>1</sup> Hertwig, O.<sup>2</sup> the writer<sup>3</sup> and others is no doubt erroneous.

The important fact, however, is that a certain definite response on the part of the developing organism may be consistently obtained after carefully adjusted treatments with a large number of different substances. Since the response is the same in each

<sup>1</sup> Herbst, C. Experimentelle Untersuchungen, u. s. w., *Zeitschr. f. wissensch. Zool.*, 4, 1892; *Mitt. a. d. Zool. Staz. zu Neapel*, 1893; *Arch. f. Entw.-Mech.* 4, 1896.

<sup>2</sup> Hertwig, O. Urmund und Spina bifida. Eine vergleichende morphologische teratologische Studie an missgebildeten Froscheiern. *Archiv f. Mikr. Anat.* Bd. 39, 1892; *Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre.* *Archiv f. Mikr. Anat.* Bd. 77, Abt. 2, 1911.

<sup>3</sup> Stockard, C. R. The artificial production of cyclopic monsters: The "Magnesium embryo." *Jour. Expr. Zool.*, 6, 1909.

case it is very probable that the substances though widely different act similarly on the embryonic organism, for example, in certain cases they may serve simply to lower the developmental metabolism and thus prevent or arrest the formation of particular structures.

The hen's egg readily lends itself to chemical and mechanical experiments and has been largely employed in experimental teratology. It has long been known that by running the incubator at too high or too low a temperature or by reducing aeration by varnishing the shell one is able to obtain a most varied group of monsters. Féré<sup>4</sup> has used a great number of methods to produce monster chick embryos. In 1899 he treated eggs with alcohol fumes before incubation and found that the fumes penetrated the shell and produced various abnormalities in the embryos. Féré<sup>5</sup> also repeated Preyer's<sup>6</sup> experiment of removing the egg from the shell and allowing it to develop in glass dishes. Preyer was only able to keep the eggs under observation in this manner for two or three days, while Féré devised a better means of ventilation and succeeded in keeping the eggs alive for six days. Many of the embryos developing out of the shell showed abnormalities. Féré's reports merely record the experiments and mention the types of monsters obtained but no detailed or systematic study was undertaken and his experiments have generally passed unnoticed. One must, however, appreciate the rather ingenious and various methods of treatment which Féré employed.

The experiments to be briefly described in the present communication are presented in order to show that the central nervous system and the eyes of the chick embryo become affected in a manner closely similar to that which I have recorded for the fish embryos when treated with alcohol, ether and other substances.<sup>7</sup>

<sup>4</sup> Féré, Ch. Influence du repos, sur les effets de l'exposition préalable aux vapeurs d'alcool avant l'incubation de l'œuf de poule. *Compt. rend. Soc. de biol.* 51, 1899.

<sup>5</sup> Féré, Ch. Remarques sur l'incubation des œufs de poule privés de leur coquille. *Compt. rend. Soc. de biol.* 52, 1900.

<sup>6</sup> Preyer, W. *Physiologie spéciale de l'embryon.* Trad. franc, p. 16, 1887.

<sup>7</sup> Stockard, C. R. The influence of alcohol and other anesthetics on embryonic development. *Am. Jour. Anat.*, 10, 1910.

Hens' eggs were exposed for different lengths of time to the fumes of alcohol and ether. The eggs used in the experiments had been laid for only two or three days. Shallow dishes were arranged with a wire screen bottom beneath which absorbant cotton soaked with 95 per cent alcohol was placed. Eggs were placed upon the wire screen and the dishes covered and left standing at the room temperature. During the two years several hundred eggs were treated in this manner.

After the eggs have been exposed to the fumes for a short while the shell becomes covered with moisture, the condensed alcohol vapour and this vapour penetrates the shell. The eggs were exposed for from twenty minutes up to thirty hours at room temperature. The shortest exposure that gave effects was three hours and forty-five minutes, though in many cases an exposure of as long as eight hours was non-effective. Exposures of from fourteen to twenty hours gave the best results. In these cases almost every embryo was abnormal yet most of them were able to continue development for several days at least. Exposures of twenty-three hours or more were usually fatal, the eggs failing to develop after being put into the incubator.

The chief point to consider in the amount of exposure is the temperature. When the temperature is high evaporation is more rapid and more alcohol enters the egg in a given time.

If eggs are placed in the incubator immediately after the treatment liquid oozes out of the pores in the shell on account of the slight expansion of the egg contents as the temperature rises. A certain amount of the alcohol is no doubt lost by this process. It is better, therefore, to allow the eggs to remain at room temperature for several hours after being removed from the fume dishes and before being placed in the incubator. Féré found that eggs put into the incubator immediately after treatment with alcohol fumes were not so decidedly affected as those treated for the same length of time but not subjected to the raised temperature until several hours after the treatment.

In other cases eggs were exposed to the alcohol fumes while in the incubator. Weak alcohol solutions were placed below the egg tray and evaporated slowly. This treatment was also



continued for different lengths of time and in many cases gave more decided effects than those obtained by the treatments before incubation.

Ether fumes were also employed in the above manner. These fumes induce the same general types of developmental abnormalities though they are more decided in action than alcohol fumes and kill the embryos much more readily.

Several injection methods were used and a number of substances were injected into the egg but the results were indefinite and often negative. In many cases the injection was a failure in that it either coagulated the albumen in the region, or injured the egg so that it did not develop.

Following effective treatments with the fumes of alcohol or ether the embryos were found to be small and behind the control in their rate of development. The abnormalities most abundant were of a general nature, in some cases the entire body of the embryo was absent while the area vasculosa was present containing blood islands and embryonic vessels. Other cases showed small embryos with the brain portion of the neural tube poorly developed. The circulation in many of the embryos was slow and sluggish and in such cases hydramnious conditions were present and the blood sinuses were also distended.

A number of the embryos showed various abnormal eye conditions and these are the defects of particular interest since exactly similar abnormalities have been gotten in abundance when developing fish eggs are subjected to the actions of ether and alcohol. In several experiments embryos occurred with small poorly formed eyes which closely resembled the minute defective eyes most commonly found in alcoholized fish embryos.

A few typical cyclopean conditions were obtained showing different degrees of the defect. However, never more than three or four per cent of the embryos showed cyclopia even in the most successful experiments and in most instances cyclopia did not occur at all. Nevertheless, it is of importance to find that these treatments do occasionally induce the same variety of defects in the chick as was so abundant in many of the fish experiments.



The monster monophthalmicum asymmetricum, that is, an individual with one eye of the normal pair perfectly developed and the other eye either absent or defective to a marked degree, was commonly seen in the different groups of embryos, plates 1 and 2. This condition was more often found than cyclopia, yet it also was not as abundant as in fish embryos developing in solutions of alcohol or ether.

The failure to obtain definite defects in large numbers in the chick embryos is no doubt due to the fact that the amount of treatment is much more difficult to regulate than in such an egg as that of the fish. The great variation in the size of hens' eggs, the amount of albumen as well as yolk, the thickness of the shell, etc., makes it almost impossible to treat a number of eggs to the same degree. The treatment must of course be delicately balanced in order to obtain such typical defects as cyclopia and monophthalmica since they only occur as responses to a certain injury or arrest at a critical developmental stage.

It has also been found in a series of experiments which is being conducted to test the effects of alcohol and ether on the structure of the offspring from guinea-pigs that a completely eyeless young animal was produced and the nervous systems of almost all the offspring show some defects due to the treatment.<sup>5</sup>

During the winter of 1912 one of the incubators in the laboratory was placed in a room into which a ventilation system opened. The same system communicated with rooms in the chemical laboratory and fumes conveyed by the ventilator although rarely noticeable in odor were sufficient to injure the developing chicks. Many of the embryos died during early stages. The eggs were being used in tissue culture experiments by Dr. Burrows and were usually opened after having developed for about twelve to eighteen days. Several of these large chicks were found to have only one lateral eye. They were similar to the early embryos formed in the above experiments and were asymmetrical monophthalmic monsters identical with those I have described in

<sup>5</sup> Stockard, C. R. The effect on the offspring of intoxicating the male parent and the transmission of the defects to subsequent generations. *Am. Naturalist*, vol. 47, 1913.

fish embryos. Photographs of three of these large chick embryos are figured in plates 1 and 2, since they illustrate the defect far better than the young three and four day twisted embryos. The fumes injured the eggs and caused the same types of developmental arrests or suppression as are obtained with the other substances discussed above. After the incubator was removed from this room the eggs in it developed in a perfectly normal manner.

These structural deformities and their experimental production are recorded to emphasize the general nature of such defects and their wide occurrence among different types of embryos when treated with any substance which tends to arrest development or lower their developmental rate and vigor. Elsewhere<sup>2</sup> I have attempted to show how all abnormalities such as these eye structures may be explained merely as developmental arrests. Thus their wide occurrence in spite of their typical appearance.

## PLATE 1

### EXPLANATION OF FIGURES

Three views of an asymmetrical one-eyed chick monster which occurred in Dr. Burrow's incubator. The upper photograph shows the eyeless side, a small nodule of skin in the orbital depression represents an abortive eye-lid formation. The lower left figure represents the opposite side with a perfect eye, the fully developed lids are closed. The lower right figure giving a dorsal view of the head emphasizes the general asymmetry due to the absence of the one eye. The beak is permanently crossed since the upper jaw is forced to incline towards the eyeless side while the lower jaw remains in a normal position. It is thus impossible to close the beak as all the figures show.

<sup>2</sup> *Am. Journ. Anat.*, vol. 15, no. 3, 1913.



## PLATE 2

### EXPLANATION OF FIGURES

Two other specimens of monster *monophthalmicum asymmetricum*. The huge eye of the embryo chick is seen on one side of the head while the other side is eyeless. Both of these embryos also show the twisted upper jaw and the permanently open condition of the mouth.







## A CASE OF PATENCY OF THE PERICARDIUM AND ITS EMBRYOLOGICAL SIGNIFICANCE

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### ONE FIGURE

During the winter of 1913 there was found in our laboratory an infrequent malformation of the pericardium in which there existed a large foramen, connecting the pericardial sac with the left pleural sac. Besides this condition there also occurred other anomalies of the coelomic derivatives, which if correctly interpreted, point back to an early disturbance in the development of the general coelomic cavity. On account of the rarity of this condition, and on account of its broad embryological significance, it was thought that the following report would not be out of place.

Briefly, the history of the case is as follows: Male, sixty-five years old; family history not obtained. After being in the Newberry State Hospital for eleven years, suffering from terminal dementia, the patient died with symptoms of gastritis. Death occurred in 1913. During his residence at the hospital no symptoms were observed which would point to the condition we are describing.

On examination of the body during the process of dissection the rare condition was found of a large pleuro-pericardial foramen. In addition to this there was also found a group of peritoneal disturbances; namely, a ventral hernia, left inguinal hernia, tendency to double femoral hernia, and malposition of the colon. They will be described in that order.

The opening between the pericardial and pleural sacs appeared as an opening from 7 to 8 cm. in diameter. The edge was free throughout its course, which extended from above the pulmo-

nary artery, thence over the root of the lung. From there it arched slightly forward, following the groove between the systemic and pulmonary aortae. At the junction of the pulmonary artery with the right ventricle the fold turned downward and backward, then upward to terminate back of the left atrium. The free edge continued laterally to the left, forming the left



Figure 1. Left pleural cavity viewed from the left side, with the left lung removed. The large foramen in the mediastinal pleura, in front of the root of the left lung opens directly into the pericardial sac, exposing the heart; 1, arch of aorta and large vessels; 2, pulmonary aorta; 3, left auricular appendage; 4, root of left lung; 5, left phrenic nerve appearing through the left mediastinal pleura; 6, diaphragmatic pleura.

parietal layer of the pericardium, and to the right, forming the right costal pleura. Through the opening could be seen the pulmonary artery and left auricular appendage, as shown in figure 1. The left phrenic nerve passed between the two layers of the anterior edge of the foramen. Aside from the large opening in it, the pericardium was normal. There were no signs of adhesions or other disease except for a few adhesions over the apices



of the lungs. The upper lobe of both right and left lungs was partially divided in each case, by a fissure from 1 to 2 cm. deep. Nothing abnormal was found regarding the diaphragm.

On examination of the peritoneum there was found a small ventral hernia, 1 cm. from the median line midway between the umbilicus and xiphoid cartilage. It pierced the transversalis fascia, both layers of the rectus sheath and the rectus muscle, appearing beneath the skin. The opening was 1 cm. in diameter. Through the opening protruded a tag-like appendage which appeared to be made up of a portion of the falsiform ligament of the liver. A small vein and artery passed into it from the internal mammary vessels. The left inguinal hernia was a very large oblique hernia extending down to the bottom of the scrotum. It contained a large fold of the great omentum. In the region of both femoral rings there were distinct short funnel-shaped pockets of the peritoneum extending into the femoral canals.

The malposition of the colon was determined by the abnormal disposition of its peritoneal reflections. The peritoneal covering was more complete than usual. Thus the caecum and part of the ascending colon were completely surrounded, and were suspended free in the cavity by a mesentery. The ascending colon was flexed ventrally and upward upon itself so that the caecum was lying above the right lobe of the liver. The vermiform appendix, 10 cm. long, was found beneath the junction of the sixth costochondral articulation, at the level of the xiphoid cartilage of the sternum. It passed down medially between the caecum and ascending loop of the sigmoid. The sigmoid colon formed a large loop with a broad mesentery. The upper limb of the flexure extended obliquely upward across the umbilical and hypogastric regions into the right hypochondrium, where it, together with the caecum, caused a marked depression upon the anterior surface of the liver. The flexure turned here and the lower limb passed downward and backward through the right hypochondrium, and from thence downward into the true pelvis, having formed a loop about 16 inches long. It was supported throughout its whole length by a mesentery. The great omen-

tum was much enlarged and formed, as has been mentioned, the contents of the left inguinal hernial sac.

On examination of the literature I have been able to find eighteen cases of defective pericardium. Three of these were found in foetuses and the remainder in adults. I have been able to examine the original articles of nine of these cases. Of the remaining nine, five were found in descriptions given by other writers, while the last four could not be utilized as the descriptions and references were either incomplete or the source unavailable. The nine cases, the accounts of which I have had access to, are as follows:

Baillie (1788) reported a condition in a male of forty, in which the heart was found to lie free in the left pleural cavity. The mediastinum consisted of two laminae of pleura, inclined to the right side of the chest. Both laminae were connected throughout their extent by the intervention of a cellular membrane. This passed over the vena cava about 1 inch above the auricle. The heart was involved in the reflection of the pericardium, which became its immediate covering. This covering was very thin. The left phrenic nerve ran between the two laminae almost immediately under the sternum.

Curling ('39) reported a case in which, upon opening the chest, the heart was found completely exposed, lying loose in the cavity of the left pleural sac. There was no appearance of any pericardium covering the heart. The only indication of a pericardium was a reflected fold which covered the pulmonary vessels on the right side. The fold on the right side, close to the diaphragm, presented a small serous pouch with defined margin inferiorly and into which the appendix of the auricle protruded. The anomaly was discovered in a male of forty-six.

Baly ('50) reported a case of malformation of the pericardium in a male aged fifty-two. The malformation was discovered during a postmortem. The heart and left lung were found to be in the left pleural sac. The heart was in close contact with the lung, but connected in no way with the diaphragm. The membrane forming the common sac constituted the pleura of the lung in one case, and the pericardium in the other. The mem-

brane continued in the horizontal direction, after leaving the sternum lined the ribs on the left side, covering the outer and posterior surfaces of the lung. On its inner surface it was reflected at the root of the lung, directly upon the pulmonary veins, thence to the right pleura. The left lung was described as being covered by a false membrane. The phrenic nerve passed in front of the arch of the aorta to reach the septum between the two pleural sacs.

Bristowe ('54) reports a peculiar pericardium found in a male of twenty-eight. The heart was much enlarged. The heart and left lung were both contained in the left pleural cavity. The lower part of the lobe of the left lung was firmly attached to the anterior surface of the left side of the heart. A fold of membrane existed at the upper right side of the heart. It commenced at the pulmonary artery, passed over the aorta and vena cava, descending to the diaphragm. From this point it was lost in the root of the left lung. The fold consisted of fibrous tissue covered on either side by pleura. It was widest at the right auricle, where it was about 1 inch in depth. The fold was adherent to the heart in several places. The right phrenic nerve took its normal course, but the left passed down between the layers of the membrane, about one-half inch from its edge.

Powell ('68) reported a case in which a foramen connecting the pericardium and left pleura was found. The communication was situated above, and anterior to the root of the lung. It was small and oval in shape, being less than 1 inch in diameter. There were no adhesions, and the pleura in general was very thin. The left lung was found collapsed, the pleura containing a little fluid. The pericardium contained some air and a little fluid, the heart being compressed backward. To all appearances the opening was a congenital one.

Bjornstrom ('71) reported an anomaly which occurred in a female of forty. Only about one-third of the right side of the heart was covered with pericardium. The remainder lay free in the left pleural sac in direct contact with the lung. A large foramen connected the pericardial and left pleural sacs. Only that portion of the parietal pericardium was found which formed



the wall between the right lung and heart. The portion which was back of the right auricle went over into the visceral leaf and surrounded the heart on the right side; from here it passed on to the sternum, where it continued as the left pleura.

Primrose ('01) reported a patency of the pericardium occurring in a male of sixty. An opening existed between the pericardium and left pleura which was about 3 inches in diameter. The structures which showed through the foramen were as follows: aorta, from its appearance to about 1 inch beyond origin of the left subclavian artery; pulmonary aorta, from its origin to its bifurcation; and the left auricular appendage. No indications of adhesions were present. A number of other anomalies were present which involved principally the genito-urinary system.

Keith ('07) reported two cases of malformation of the pericardium. The first case of deficiency of the pericardium was found in an anencephalic full-term child. The opening, just anterior to the root of the left lung, was about 1 inch in diameter, and through which the left auricular appendage protruded. It had a round smooth margin. The phrenic nerve descended in the anterior free edge of the foramen.

The other case occurred in a foetus, the subject of numerous malformations. It presented a large deficiency on the left side. Upon removal of the sternum a strong fibrous membrane was found behind it, upon which the phrenic nerve descended. This proved to be the pericardium, which descended and divided, the left margin passing in front of and below the left lung. Turning back at the lower margin, it appeared as a fold extending up from the diaphragm. The greater part of the left pleural cavity being occupied by the liver, stomach and spleen.

The five cases to which reference has been made by other writers included one in which the heart was found lying free in the left pleural cavity, devoid of pericardium, that was reported in the *Philosophical Transactions*, London, 1740, and referred to by Baillie in 1788. The same author refers to similar cases recorded by Columbus, Bartholinus, and Littre, in which no details were mentioned. Peacock ('68), in his work on the malformations of the human heart, refers to a case by M. Breschet ('26), in which the



absence of the pericardium occurred in a male of twenty-eight. Another case was reported by Hud in 1848. He also mentioned a specimen in the St. Thomas Museum, London. Peacock mentions a case found by himself in a man of seventy-five. He, however, did not describe it. There were three cases of malformation of the pericardium for which I have not been able to get the original articles, the titles of which are given in the references at the end of this paper.

Not including my own case, we may summarize the literature of the malformation of the pericardium as follows: (a) two cases of supposed complete absence of the pericardium; (b) seven cases of incomplete pericardium, which is represented by a small fold of tissue along the posterior wall; (c) three other cases of incomplete pericardium, in which existed a distinct opening between the left pleural sac and pericardial sac, varying in diameter from less than one-half inch to over 3 inches; in six cases the condition was not definitely described, the only mention made being of both heart and lung lying in the left pleural sac.

The only attempt by any of the writers to explain these cases on an embryological basis, was made by Keith. This writer attributed the patency to, "an extension of the lung bud growing into and expanding the communication between the pericardium and pleura."

Peacock thought that the pericardium developed as a continuation of the fibrous sheath of the vessels of the heart, which spread out over the heart, and formed its sac. He considered the foramen as due to a failure of fusion of the membrane on the left side.

None of the previous writers directed their attentions to the related serous cavities, and no examination was reported, of the peritoneum and its appendages.

Before entering into the embryological significance of the malformations it may be well to give a brief review of our present knowledge of the development of the coelom. It was early shown by His that the body cavity in the early embryo is divided into the pericardial and trunk cavities. The communication between these spaces is called the parietal recess. The parietal

portion originally contains the heart, and is destined to become the pericardial coelom. A portion of the parietal recess forms the pleural cavity; it surrounds the lung bud throughout its development, and becomes the pleural coelom. In the remainder of the parietal recess the liver and stomach develop, but are later evaginated and become part of the abdominal coelom.

For our knowledge of the details of the separation of these cavities we are indebted to Mall. He showed that at about the end of the fifth week, while the body is yet kinked upon itself, the line of separation appears between the pericardial and pleural coeloms. This is due to a constriction of the walls along the ductus Cuvieri, which lies on a ridge of tissue encircling the canal of communication between the two cavities. This forms the beginning of the pulmonary ridge. This ridge appears as a small elevation, in the sagittal plane of the body, running from the lobe of the liver, along the dorsal wall of the ductus Cuvieri, to the dorsal attachment of the mesocardium. Lying in the sagittal plane of the body opposite the fourth and fifth cervical nerves it receives into its substance the phrenic nerve, which passes posterior to the ductus Cuvieri.

Soon the lung bud, which has heretofore hung free in the pleural coelom beneath the pulmonary ridge, grows outward against it and causes it to bulge. With the rotation of the liver towards the head the ridge is divided into two parts: (1) the cephalic which has included in it the phrenic nerve, and ductus Cuvieri, and which later becomes the pleuro-pericardial membrane; (2) the caudal portion, which remains at the caudal end of the septum transversum and liver, on the one hand, and the body wall on the other. It later forms the pleuro-peritoneal membrane.

The pulmonary ridges from their beginning to their separation into the pleuro-pericardial and pleuro-peritoneal membranes appear as two ear-like projections from the septum transversum, extending along the ductus Cuvieri. They appear in the sagittal plane of the body at right angles to the plane of the septum transversum. The growth of the pleuro-pericardial membrane in the direction of the head and the growth of the pleuro-peritoneal membrane caudally results in a widening of the dorsal projection

of the septum transversum. The lung burrows into this space throwing the pleuro-cardial membrane and phrenic nerve to its medial side. Up to this time there has been a mere slit where the pleuro-pericardial membrane comes in contact with the root of the lung. At the time of closure the small ridge or pleuro-pericardial membrane, is very insignificant, its extension being due to a rapid growth of the lung.

Brachet showed that the canal connecting the cavities was only constricted by the ductus Cuvieri, its complete closure being due to an active growth of the anlage of the pleuro-pericardial membrane, which takes place at about this time. This completely separates the pericardial from the pleural cavities. Immediately after this the rotation of the liver and setum transversum takes place which changes the relation of the pleuro-pericardial membrane from parallel to right angles to it. By this time the pleuro-peritoneal membrane stretches across the body to the tips of the embryonic ribs, thus completely closing off the abdominal cavity. This also alters the position of the phrenic nerve.

With the steps of the development of the coelom in mind, we are in position to understand something of the manner of occurrence of defects in the pericardium and other coelomic derivatives. In my own case it is evident that there was a general involvement of the coelom. We are not accustomed to thinking of pathologic processes in the embryo limited to the developing coelom, but it is evident that such must exist. It is well known that the neural plate passes through a period when it is particularly sensitive to injury while the adjacent tissues are unaffected, and thus we have a group of pathological conditions, as anencephaly, spina bifida, etc., that date from this period. In a similar way it is reasonable to suppose that the cells lining the coelomic space, may at some period be particularly sensitive, and abnormal conditions occurring at this time, would result in disturbances, either an over production or an under production, of the serous derivatives. Thus we might naturally expect congenital hernias, gastropnoxis, enteropnoxis, and other abnormal conditions of the peritoneum, occurring at the same time with abnormal conditions of



the pericardium and pleura, which condition is well illustrated in our case. The occurrence of a patent pericardium is one aspect of a general condition. It is possible also in our case that in the process of subdivision of the general coelomic spaces an undue proportion was constricted off by the lower limb of the pulmonary ridge, resulting in an over production of peritoneum, and an under production of thoracic serous membrane.

Those cases in which a foramen occurred, including the one found in our laboratory, between the pleura and pericardium seem to be explained by supposing that in the early development of the embryo, some slight injury occurred to the general coelom, which resulted in a lack of development of the pleuro-pericardial membrane. The membrane, which was to form the wall between the heart and lung failed to fuse with the root of the lung bud, and the pleuro-pericardial foramen resulted. This view is also supported by the position of the phrenic nerve.

The explanation given by Keith ('06), according to whom the foramen was due to the presence of the lung which kept the communication between the pleural and pericardial cavities open, could hardly be the cause, as the lung bud forms subsequent to the development of the fold, which separates the cavities.

The case in which only a small portion of the supposed pericardium was found, existing as a ridge or fold at the base of the heart, seem to be readily explained. It at once suggests itself that the condition, with which we were dealing, was due to a less complete separation of the pleuro-pericardial membrane than occurred in those cases presenting a foramen. Thus the heart and lung would lie in a sac, which if it had separated, would have formed the pericardium and pleura, the fold or ridge of membrane existing at the base of the heart being the embryonic remains of the upper portion of the pulmonary ridge. The phrenic nerve in these cases was found under the sternum, probably never having been included in the substance of the pulmonary ridge.

Those cases in which a total absence of the pericardium was supposed to have occurred are explained as follows: The upper limb of the pulmonary ridge totally failed to develop. The con-



dition there is apparent. The heart and lung lie in one sac, which if correctly named would be pericardial, inasmuch as the left pleural sac had never become separated off. These cases must not be confused or connected with the cases described by Todd ('13) and others regarding the absence of the pleural sac in certain mammals. In those cases the pleural sacs were originally present, but in later life became obliterated.

The second case reported by Keith ('07) forms an interesting variation. Here the lung, heart and liver were found all occupying the same cavity. This condition must be explained by the involvement of both the pleuro-pericardial and pleuro-peritoneal membranes. As has been noted, the pericardial defects always are found on the left side. This apparently is associated with the asymmetry of the liver, and its rotation during the course of development, which would put a greater tension on the left pulmonary ridge, and predispose this to the defect.

Before concluding I wish to express my obligations to Professor Streeter at whose suggestion this report was undertaken.

#### CONCLUSIONS

1. Pericardial defects result from a disturbance occurring between the fifth and seventh weeks of embryonic life.
2. These defects always occur on the left side.
3. Other coelomic disturbances of the same period occur in the form of peritoneal abnormalities, such as congenital hernia, gastropnoxis and other abnormal arrangements, and distributions of the peritoneum.
4. Congenital pericardial defects have not yet been clinically diagnosed and apparently produced no functional disturbance.

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## THE PERCENTAGE OF WATER IN THE BRAIN OF THE SMOOTH DOG-FISH, *MUSTELUS CANIS*

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Donaldson<sup>1</sup> has shown that in the albino rat between birth and maturity the percentage of water in the brain diminishes from 87.8 per cent to 77.5 per cent. He calls attention to the fact generally known that the human brain at birth contains a greater percentage of water than at maturity and from the investigations of Weisbach and Koch he obtains as the percentage of water in the human encephalon the following: birth, 88.3 per cent; two years, 81.1 per cent; five years, 79.2 per cent; twenty-five years (mature), 77.0 per cent. Donaldson further says:

We reach the interesting conclusion that probably in all mammals we shall find approximately the same range in the percentage of water between birth and maturity and that the loss of water in them occurs in the same manner but that the time required for each successive step is determined by the intensity of the growth process characteristic for each species.

The present author in 1910 had obtained the percentage of water in the brain of a few smooth dog-fish but at the suggestion of Dr. Donaldson, has collected further data on this subject in order to see whether the above law holds true for the elasmobranchs, which occupy a place at the base of the vertebrate ladder.

The author collected the following data at the Biological Laboratory of the United States Bureau of Fisheries at Woods Hole, Massachusetts. He is greatly indebted to the Bureau of Fisheries for the material and facilities furnished him.

The data are not as complete as they might be but since they illustrate a difference between the elasmobranchs and the mam-

<sup>1</sup> Donaldson, H. H., Jour. Comp. Neur., vol. 20, no. 2, p. 119, April, 1910.

mals, this paper is presented at this time. The percentage of water in the brain of ninety-seven smooth dog-fish, *Mustelus canis*, was obtained. These were obtained from the laboratory trap in Buzzard's Bay and the brain tissue was removed on the same day that the fishes were brought into the laboratory. In

TABLE 1

*Showing the percentage of water in the brain of the dog-fish, Mustelus canis, of increasing body length. Sex, male.*

NUMBER	LENGTH	BRAIN WEIGHT	WATER IN BRAIN	NUMBER	LENGTH	BRAIN WEIGHT	WATER IN BRAIN
	cm.	grams	per cent		cm.	grams	per cent
1	39	1.39	77	27	70	2.60	79
2	42	1.42	77	28	70	2.61	79
3	42	1.48	77	29	72	3.17	81
4	44	1.41	77	30	74	3.36	78
5	45	1.51	79	31	75	3.18	78
6	52	1.98	79	32	75	3.20	78
7	55	2.08	79	33	75	3.20	78
8	56	2.24	74	34	76	3.14	78
9	57	2.26	80	35	77	3.52	79
10	60	2.24	80	36	77	3.55	80
11	60	2.33	77	37	77	5.77	85
12	61	2.35	77	38	79	3.44	74
13	64	2.50	82	39	79	3.41	78
14	65	2.13	74	40	80	3.45	75
15	65	2.67	79	41	80	3.33	80
16	65	2.80	79	42	81	3.78	80
17	65	2.61	79	43	81	4.16	81
18	66	2.59	80	44	82	3.38	79
19	67	2.79	81	45	82	3.25	74
20	67	2.91	79	46	82	3.65	78
21	67	3.35	81	47	82	3.65	78
22	69	2.94	80	48	83	3.47	80
23	69	3.17	79	49	85	3.60	80
24	70	2.77	75	50	90	4.06	81
25	70	3.05	77	51	91	3.89	79
26	70	2.94	77				

each case the following technique was employed. The sex, length and weight of each specimen was first recorded, then the brain case was opened. The olfactory tracts were severed close to the forebrain, a transverse cut made at the posterior margin of the fourth ventricle, the cranial nerves severed and the brain



carefully placed on clean filter paper. A longitudinal cut was then made through the brain and each half very carefully turned over on the filter paper until no further cerebral fluid was absorbed. The brain tissue was then placed in a watch crystal and its weight determined. It was next placed in a desiccator over sulphuric acid. The desiccator was made a partial vacuum.

TABLE 2

*Showing the percentage of water in the brain of the smooth dog-fish, Mustelus canis, of increasing body length. Sex, female.*

NUMBER	LENGTH	BRAIN WEIGHT	WATER IN BRAIN	NUMBER	LENGTH	BRAIN WEIGHT	WATER IN BRAIN
	cm.	grams	per cent		cm.	grams	per cent
1	42	1.48	78	24	75	3.27	76
2	44	1.33	78	25	75	3.35	79
3	45	1.47	78	26	75	3.02	83
4	56	2.20	77	27	75	3.63	79
5	60	2.26	79	28	77	3.48	82
6	62	2.40	78	29	77	3.88	84
7	62	2.48	80	30	79	3.58	75
8	62	2.42	80	31	79	3.51	80
9	62	2.24	77	32	80	3.24	75
10	62	2.53	78	33	81	3.29	81
11	62	2.73	79	34	81	3.27	84
12	62	2.92	80	35	82	2.85	80
13	66	2.65	76	36	82	3.56	79
14	66	3.20	81	37	82	3.50	78
15	67	2.73	79	38	89	3.82	78
16	69	2.99	79	39	90	4.22	80
17	69	2.55	79	40	90	3.74	80
18	70	3.33	76	41	92	4.26	78
19	71	2.92	77	42	96	4.11	77
20	72	3.25	77	43	97	4.28	79
21	74	3.22	76	44	99	4.58	77
22	74	3.31	81	45	104	4.45	76
23	75	3.49	78	46	105	4.49	78

The brain tissue was then dried to a constant weight and the percentage of water computed. Since in this problem the percentage of water only was desired, the same great care to get every trace of brain tissue was not as necessary as in the case where the exact weight of the brain at various ages was to be investigated.

The change in the weight of the brain of *Mustelus canis* and of increasing body weight has been carefully worked out by Kellicott ('08) whose paper will be referred to later. Tables 1 to 5 show the results obtained. Tables 1 and 2 show the percentage of water in the brain of smooth dog-fishes of increasing body

TABLE 3

*Showing the percentage of water in the brain of the smooth dog-fish, Mustelus canis, of increasing body weight. Sex, male.*

NUMBER	WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	NUMBER	WEIGHT	BRAIN WEIGHT	WATER IN BRAIN
	grams	grams	per cent		grams	grams	per cent
1	218	1.39	77	27	1057	3.05	77
2	264	1.48	77	28	1057	3.55	80
3	280	1.42	77	29	1057	2.80	79
4	311	1.51	79	30	1088	5.77	85
5	326	1.41	78	31	1120	2.79	81
6	420	1.98	79	32	1244	3.20	78
7	451	2.08	79	33	1306	3.18	78
8	560	2.26	80	34	1337	3.20	78
9	560	2.61	79	35	1368	3.14	78
10	575	2.24	74	36	1399	3.36	78
11	591	2.33	77	37	1399	4.16	81
12	653	2.35	77	38	1462	3.44	74
13	669	2.24	80	39	1462	3.38	79
14	684	2.91	79	40	1462	3.33	80
15	715	2.50	82	41	1555	3.41	78
16	746	2.13	74	42	1586	3.78	80
17	746	2.67	79	43	1648	2.60	80
18	746	3.35	81	44	1679	3.25	74
19	775	3.71	81	45	1679	3.52	79
20	840	3.06	80	46	1773	3.45	75
21	933	2.59	80	47	1773	3.47	80
22	933	3.17	79	48	1990	4.06	81
23	995	2.77	75	49	2021	3.65	78
24	995	2.94	77	50	2053	3.65	77
25	995	2.60	79	51	2379	3.89	79
26	1042	2.94	80				

length, males and females respectively. Tables 3 and 4 show the percentage of water in the brain of fishes arranged according to increasing body weight instead of length.

Donaldson found that at different body weights the male brain contains a greater percentage of water than the female

brain in the case of the albino rat. Not only is this sex difference true of the percentage of water, but as is commonly known, the male brain actually weighs more than the female brain in relation to the weight of the body. Kellicott,<sup>2</sup> on the other hand, found that in the smooth dog-fish there was no sex difference as to total brain weight. He says, "It is not possible to distinguish

TABLE 4

Showing the percentage of water in the brain of the dog-fish, *Mustelus canis*, of increasing body weight. Sex, Female.

NUMBER	WEIGHT	BRAIN WEIGHT	WATER	NUMBER	WEIGHT	BRAIN WEIGHT	WATER
	grams	grams	per cent		grams	grams	per cent
1	280	1.48	78	24	1151	3.48	82
2	295	1.33	78	25	1182	3.31	79
3	342	1.47	78	26	1213	2.73	79
4	529	2.20	77	27	1244	3.49	78
5	560	2.92	80	28	1368	3.27	76
6	622	2.73	79	29	1368	3.50	78
7	637	2.26	79	30	1368	3.51	80
8	653	2.42	80	31	1493	3.22	76
9	684	2.40	78	32	1550	3.58	75
10	715	3.20	81	33	1617	3.88	84
11	746	2.24	77	34	1679	3.29	81
12	746	2.55	80	35	1679	2.85	80
13	762	2.48	80	36	1835	3.24	75
14	809	2.53	78	37	1928	3.56	79
15	871	2.65	76	38	2364	3.74	80
16	871	3.35	79	39	2395	3.82	78
17	902	3.33	76	40	2581	4.22	80
18	964	2.99	79	41	2846	4.58	77
19	1026	2.92	77	42	2892	4.26	78
20	1057	2.55	79	43	3297	4.11	77
21	1057	3.02	83	44	3390	4.45	76
22	1057	3.63	79	45	3452	4.28	79
23	1151	3.25	77	46	4198	4.49	78

between the sexes with respect to brain weight." But what is the condition as regards the percentage of water? The average percentage of water in the brain of the forty-six females recorded here is 78.6 per cent, while that of the fifty-one males is 78.5 per cent. There is no sex difference in *Mustelus canis* as far as the

<sup>2</sup> Kellicott, W. E., Am. Jour. Anat., vol. 8, no. 4, p. 207, December, 1903.

percentage content of water in the brain tissue goes. This is in agreement with the result obtained by Kellicott.

But what is the condition as regards the percentage of water in the brain at different ages?

Since there are no sex differences we can group together the males and females. Nothing is known of the exact age of the dog-fish but in general they increase in length and weight as they grow older. Kellicott, following the methods of Moenkhaus and Fulton with teleosts, has roughly estimated the ages as shown in table 5.

TABLE 5

	WEIGHT	LENGTH
	grams	cm.
Birth.....	75	32.5
1 year.....	300	45.0
2 years.....	775	63.0
3 years.....	1400	78.5
4 years.....	2325	90.0
5 years.....	2750	99.0

Now since we are ascertaining the relation of the percentage of water in the brain to the age and since age is measured by length and weight, it is necessary to distribute the specimens concerning which we have records, according to the above schedule. Applying Kellicott's criterion we have table 6.

TABLE 6

	(A) LENGTH MALE + FEMALE	(B) WEIGHT MALE + FEMALE
1 year ±.....	6 + 3 = 9	7 + 4 = 11
2 years.....	22 + 15 = 37	23 + 18 = 40
3 years.....	20 + 19 = 39	18 + 14 = 32
4 years.....	3 + 4 = 7	4 + 3 = 7
5 years.....	0 + 5 = 5	0 + 7 = 7
	Total, 97	Total, 97

We thus see that we have about the same distribution by length as by weight. It will be noted that the medium sized



are most numerous. The average percentages of water in the brains of the various groups just given are shown in table 7.

TABLE 7

	LENGTH	WEIGHT	AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1 year.....	77.8	77.9	77.8
2 years.....	78.5	78.7	78.6
3 years.....	78.9	78.8	78.8
4 years.....	79.4	79.0	79.2
5 years.....	77.4	77.9	77.7

As far as the above results go, there is no great decrease in the percentage of water with increasing age. This appears contrary to what Donaldson has found in the case of the albino rat. There he found a decrease of water of 10 per cent between birth and maturity.

I was able to secure seventeen specimens of young *Squalus acanthias*, the spiny dog-fish. These fish, as may be seen in table 8, are all under one year of age.

TABLE 8

*Showing sex, length, weight, brain weight and percentage of water in the brain of young Squalus acanthias*

NUMBER	SEX	LENGTH	WEIGHT	BRAIN WEIGHT	WATER IN BRAIN
		<i>cm.</i>	<i>grams</i>	<i>grams</i>	<i>per cent</i>
1	♂	27	140	0.60	82
2	♀	29	140	0.71	82
3	♀	30	140	0.73	82
4	♀	30	109	0.72	82
5	♀	30	140	0.81	81
6	♀	30	187	0.81	81
7	♂	31	109	0.67	84
8	♀	31	124	0.71	82
9	♀	31	93	0.79	84
10	♀	31	155	0.60	80
11	♀	32	187	0.95	83
12	♀	34	124	0.87	79
13	♀	34	124	0.94	79
14	♂	34	187	1.06	81
15	♀	35	233	0.87	80
16	♀	36	155	0.97	80
17	♀	36	171	1.06	82

Moreover, eight females and two males from the standpoint of length would be regarded as recently born, according to the age criterion given above. But when we look at the weights we find these to be much greater than what is called for, namely, 75 grams. And yet it must be remembered that we are now discussing a species other than that for which Kellicott constructed his age table.

The average percentage of water in the brain of these seventeen *Squalus acanthias* is 81.4 per cent. On the whole this group is smaller in length and weight and so younger than the smooth dog-fishes, *Mustelus canis*. There is some slight indication then of a small decrease in the percentage of water in the brain between birth and the first year. This should not be emphasized, however, since we are dealing with two different species of fishes. Kellicott has shown that during the period of which we have data, the brain of *Mustelus* has increased from about 1.5 grams in weight to about 4.0 grams. During this time also it has decreased from about 0.6 per cent to 0.2 per cent of the total body weight. And yet we have seen that the percentage of water in the brain has remained quite constant. How can we account for this?

Mammals are characterized by determinate growth. As soon as maturity is reached the organs have reached their size limit. For example, the bones increase in length no further. On the other hand, fishes have indeterminate growth, that is, they grow as long as they live. As far as the brain is concerned, in the case of mammals growth is very rapid during the first few months. On the other hand, in fishes the brain grows steadily as long as the animal lives. As Kellicott says, "After birth (smooth dog-fish) the brain weight increases rapidly but at a slightly diminishing rate. Among the large individuals the diminution is much slower but is continued during life. Donaldson shows that the diminution in percentage of water is most rapid during the first thirty days of the albino rat's life, that is, when the central nervous system is growing most actively. Amphibians also possess indeterminate growth. Tigerstedt,<sup>2</sup> reviewing the work of

<sup>2</sup> Tigerstedt, Textbook of human physiology, 1906, p. 574.

Birge, says that he "counted the motor cells in the spinal cord and nerve fibres in the anterior spinal roots in frogs of different sizes" and convinced himself "that both either multiply from preëxisting nerve elements or from other elements throughout life." He found "unmistakable relation between the weight of the animal and the number of cells and fibres. On the average for each 1 gram increase in weight, 52 motor fibres had been added."

The most significant difference between the rat and the dog-fish, as far as our present discussion is concerned, is the post-birth condition of the two. The rat is born helpless, blind and cannot move about for some time. On the other hand, the dog-fish is born, free—swimming, active and apparently mature with the exception of the reproductive system. Donaldson shows a correlation between the period of rapidly forming nerve cells and the percentage of water in the brain. Very possibly the dog-fish has a greater percentage of mature nerve cells at birth than the rat. We should expect a smaller percentage of water than in the case of the rat. This is borne out by the conditions in the young spiny dog-fishes discussed above. If the discoveries of Birge are correct and apply equally well to the dog-fishes, as we have considerable reason to believe, then the continued constancy in the percentage of water in the elasmobranch brain is due to the multiplication of new nerve cells and fibres keeping pace with the growth of the brain in other respects.

According to Donaldson's table, about seven-tenths of the percentage decrease in water takes place in the first one-eighth of the rat's life, between birth and maturity. There is a decrease of only three-tenths during the remaining seven-eighths of this maturing period, that is, it occurs during the first thirty out of the total two hundred and forty days. The period of greatest loss in water is that during which profound neurological changes take place. May not these changes take place in the dog-fish in utero? The two cases make a strong argument for considering the change in water content of the central nervous system to be correlated with the growth intensity of this system. And that in the dog-

fish the greatest change takes place in-utero, while in the rat and man it is extra-utero. The collection of data from the brains of embryonic stages is necessary to decide this hypothesis.



# A NOTE ON THE PRESENCE OF A MUSCULUS CLEIDO-ATLANTICUS IN THE DOMESTIC CAT (*FELIS DOMESTICA*)

RANDOLPH WEST

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## ONE FIGURE

So far as is known to the writer the musculus cleido-atlanticus (Gruber)<sup>1</sup> has never been described as occurring in the cat, nor in any other mammal in which the clavicle is rudimentary. In order to avoid confusion, the nomenclature used by Reighard and Jennings<sup>2</sup> will be followed throughout this paper, and, in addition, the term cleido-atlanticus will be used to designate a muscle, hitherto not described in the cat, arising from the atlas and inserting into the clavicle.

Both the m. levator scapulae ventralis (levator claviculae) which arises from the atlas and inserts into the metacromion, and the m. cleido-atlanticus have been described as anomalies in man by Testut,<sup>1</sup> under the common name of the m. cleido-omo-transversaire and by Le Double,<sup>3</sup> under the common name of the m. omo-trachélien. The m. cleido-atlanticus is found alone in the anthropoid apes, as well as in *Nycticibus tardigradus* and *Cynocephalus anubis*, while it is present in connection with the m. levator scapulae ventralis in the orang. Always one and occasionally both of these muscles occur regularly in all vertebrates except the fishes, birds and man. In some vertebrates these muscles may have origins from the basi-occipital and from the posterior cervical vertebrae in addition to the atlantal origin. For a fuller account of the comparative anatomy

<sup>1</sup> Testut, *Les anomalies musculaires chez l'homme*. 1884, p. 97.

<sup>2</sup> Reighard and Jennings, *Anatomy of the cat*. 1901.

<sup>3</sup> Le Double, *Variations du système musculaire de l'homme*. 1897. T. 1, p. 235.

of the m. cleido-atlanticus the reader is referred to the articles of Testut and Le Double cited above.

The m. cleito-atlanticus (4, fig. 1) was found in one adult female cat and was present on both sides of the body. Out of some four hundred cats dissected in the laboratory this is the

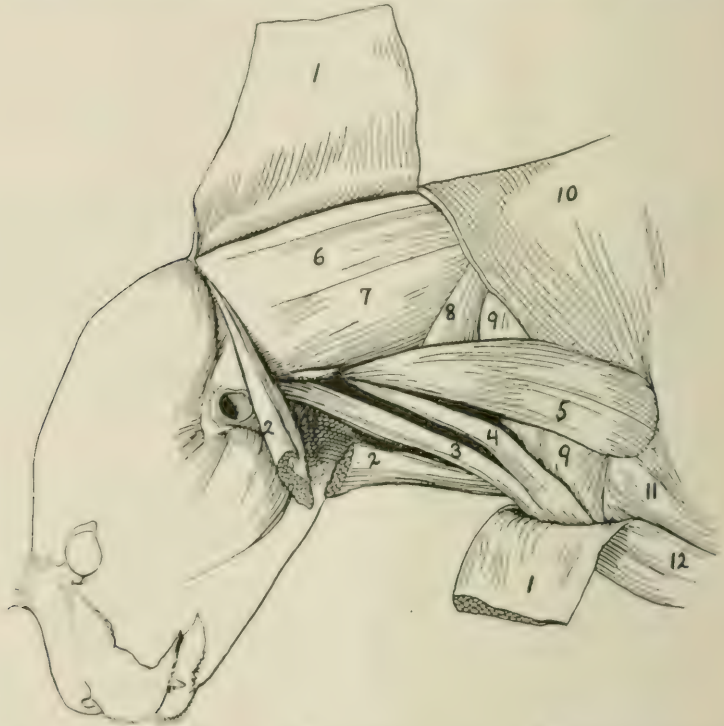


Fig. 1 1, M. clavotrapezius; 2, M. sternomastoideus; 3, M. cleidomastoideus; 4, M. cleido-atlanticus; 5, M. levator scapulae ventralis (levator claviculae); 6, M. occipitoseapularis (levator scapulae dorsalis); 7, M. splenius; 8, M. levator scapulae; 9, M. supraspinatus; 10, M. acromiotrapezius; 11, M. acromiodeltoideus; 12, M. clavobrachialis.

first one in which this muscle has been observed. It arises in common with the m. levator scapulae ventralis (5) from the posterior portion of the transverse process of the atlas. After about 0.5 cm. the two muscles separate, and the m. levator scapulae ventralis inserts into the metaacromion. The m. cleido-

atlanticus inserts into the lateral third of the clavicle and into the raphe which lies lateral to the clavicle, between the m. clavo-trapezius (1) and the m. clavobrachialis (12). About 1.75 cm. before its insertion it is joined on its medial border by the m. cleidomastoideus (3), which inserts into the medial two-thirds of the clavicle. The m. cleido-atlanticus is an elongated muscle, somewhat flattened at the clavicular end. It is about 6 cm. in length, 0.25 cm. broad at the atlas, 1 cm. broad at the clavicle and 0.5 cm. thick in the cat here described. The innervation is from the ventral ramus of the third cervical nerve which also supplies the m. levator scapulae ventralis, the m. cleidomastoideus, the m. sternomastoideus (2) and several other muscles of this region.

## ANTI-VIVISECTION MORALS

It makes no difference to an anti-vivisectionist how hard a blow she receives from the facts. She comes up smiling just the same. Dr. Keen, the famous Philadelphia surgeon, exposed recently a number of the latest lies, and Mrs. Henderson, vice-president of the American Anti-Vivisection Society, came back with the most cheerful and unmoved assertion of her own opinion and interpretation against overwhelming evidence. Then comes along Dr. Crile. Mrs. Henderson had quoted Dr. Crile's book on "Surgical Shock," saying that it "repeatedly describes experiments followed by the words 'no anesthesia.'" Dr. Crile has studied his own book faithfully, and cannot discover any such words. We have not yet noticed Mrs. Henderson's answer to Dr. Crile, but feel sure that it will be just as cheerful as her answer to Dr. Keen. *Harper's Weekly*, January 24, 1914.

## SCIENCE AND MERCY

The Anti-vivisectionists have been putting out a circular in Philadelphia, with the statement that Dr. George W. Crile made experiments on one hundred and forty-eight dogs "in an endeavor to learn the extent of the agony that can be inflicted on a living animal." Do the kind-hearted women who are backing this movement believe that Dr. Crile did anything of the sort? When they leave out all mention of anaesthesia, do they do it by accident? Surgeons until recently thought that when a patient was unconscious they could tear loose adhesions and manipulate tissues roughly without doing mischief. Crile's experiments were to determine whether this view was correct. He found that it was not; that serious injury could be caused by shock even when there was no consciousness. Realizing the difference between psychic shock, which is prevented by anaesthesia, and traumatic shock, which is not prevented by anaesthesia, is an important step ahead, which has already resulted in a lower death rate and a shorter time for recovery. Crile, like other men of science who are called monsters of cruelty by these kind but ignorant sentimentalists, is the apostle of gentleness. *Harper's Weekly*, January 31, 1914.



# PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

## THIRTIETH SESSION

*At the University of Pennsylvania, Philadelphia, Pa., December 29,  
30, and 31, 1913*

MONDAY, DECEMBER 29, 10.30 A.M.

The thirtieth session of the American Association of Anatomists was called to order by President Ross. G. Harrison, who appointed the following committees:

*Committee on Nominations:* J. Playfair McMurrich, chairman: Robert R. Bensley, Henry McE. Knowler.

*Auditing Committee;* Harry B. Ferris, chairman: Burton D. Myers.

TUESDAY, DECEMBER 30, 12.00 M. ASSOCIATION BUSINESS MEETING, PRESIDENT ROSS G. HARRISON PRESIDING.

The Secretary reported that the minutes of the Twenty-Ninth Session were printed in full in *The Anatomical Record*, volume 7, number 3, pages 91 to 98, and asked whether the Association desired to have the minutes read as printed. On motion, seconded and carried, the minutes of the Twenty-Ninth Session were approved by the Association as printed in *The Anatomical Record*.

Harry B. Ferris reported for the Auditing Committee as follows: The undersigned Auditing Committee has examined the accounts of Dr. G. Carl Huber, Secretary-Treasurer of the American Association of Anatomists and finds same to be correct with proper vouchers for expenditures and bank balance on December 26 of \$213.03. (Signed) HARRY B. FERRIS, BURTON D. MYERS; Philadelphia, December 30, 1913.

The Treasurer made the following report for the year 1913:

Balance on hand December 29, 1912.....	\$318.08	
Receipts from dues, 1913.....	1315.39	
	<hr/>	
Total deposits for 1913.....	\$1633.47	\$1633.47
Expenditures for 1913:		
Expenses of Secretary-Treasurer, Cleveland Meeting.....	\$20.50	
Postage.....	40.00	
Printing (\$11.90), typewriting (\$8.75), envelopes (\$2.50)....	23.15	
To 297 subscriptions to 1 volume of the American Journal of Anatomy and 1 volume of the Anatomical record @ \$4.50..	\$1336.50	
To exchange for foreign draft.....	.29	
	<hr/>	
Total.....	\$1420.44	\$1420.44
	<hr/>	
Balance.....		\$213.03
Balance on hand, deposited in the name of the American Association of Anatomists in the Farmers and Mechanics Bank, Ann Arbor, Michigan, December 26, 1913.		

On motion of George S. Huntington the reports of the Auditing Committee and the Treasurer were accepted and adopted.

The Committee on Nominations through its Chairman, J. Playfair McMurrich, placed before the Association the following names: President, G. Carl Huber; Vice President, Frederic T. Lewis; Secretary-Treasurer, Charles R. Stockard. For members of the Executive Committee for term expiring in 1917, Warren H. Lewis and C. Judson Herrick.

On motion the Secretary was instructed to cast a ballot for the election of the above-named officers.

Moved by J. Playfair McMurrich, seconded by Robert R. Bensley; "That this Association accepts with regret the resignation of Dr. G. Carl Huber from the office of Secretary-Treasurer and desires to place on record its high appreciation of his services and its recognition of the prominent part he has taken in bringing the Association to its present prosperous condition and in advancing the cause of Anatomy on this continent both by precept and example." Carried.

The Secretary presented the following names, recommended by the Executive Committee, for election to membership in the American Association of Anatomists:

- EDWIN A. BAUMGARTNER, Instructor in Anatomy, *University of Minnesota*.  
 HENRY BAYON, Associate Professor of Anatomy, *Tulane University*.  
 THOMAS H. BRYCE, Professor of Anatomy, *University of Glasgow*.  
 FELIX P. CHILLINGWORTH, Assistant Professor of Physiology and Pharmacology  
*Tulane University*.  
 ELEANOR L. CLARK, Research Worker, *Johns Hopkins Medical School*.  
 GEORGE W. CORNER, Assistant in Anatomy, *Johns Hopkins University*.  
 ROBERT S. CUNNINGHAM, *Johns Hopkins Medical School*.  
 A. CAMPBELL GEDDES, Professor of Anatomy, *McGill University, Montreal*.  
 STACY R. GUILD, Instructor in Histology, *Dept. Med. and Surg., University of Michigan*.  
 G. V. ARIENS KAPPERS, Director of the *International Central Institute for Brain Research of Holland*.  
 HOWARD S. MURPHY, Professor of Anatomy and Histology, Ames, Iowa.  
 D. A. RHEINHART, *Indiana University*.  
 ARTHUR ROBINSON, Professor of Anatomy, *University of Edinburg*.  
 KATHERINE JULIA SCOTT, *Johns Hopkins Medical School*.  
 PAUL G. SHIPLEY, Assistant in Anatomy, *Johns Hopkins University*.  
 R. W. SHUFELDT, Major Medical Corps, U.S.A. (Retired).  
 G. ELLIOTT SMITH, Professor of Anatomy, *Victoria University, Manchester, England*.  
 PERRY G. SNOW, Professor of Anatomy, *University of Utah*.  
 JOHNSON SYMINGTON, Professor of Anatomy, *Queens University, Belfast, Ireland*.  
 ARTHUR THOMSON, Professor of Anatomy, *University of Oxford, England*.  
 JACOB THORKELSON, Professor of Anatomy, *College of Physicians and Surgeons Baltimore, Md.*  
 RANDOLPH WEST, School of Medicine, *Columbia University, New York City*.  
 JAMES THOMAS WILSON, Professor of Anatomy, *University of Sydney, Australia*.

On motion of E. A. Spitzka, the Secretary was instructed to cast a ballot for the election of all the candidates proposed by the Executive Committee. Carried.

George S. Huntington proposed the following amendment to the Constitution. To substitute for the last sentence of Article II the following:

"These officers shall be elected by ballot at the annual meeting of the Association, and their official terms shall commence with the close of the Annual Meeting."

"At the annual meeting next preceding an election the President shall name a Nominating Committee of three members. This Committee shall make its nominations to the Secretary not less than two months before the annual meeting at which the election is to take place. It shall be the duty of the Secretary to mail the list to all members of the Association at least one month before the annual



meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting."

This proposed amendment to the constitution becomes a matter of record for this meeting and will be acted upon in due form at the next annual meeting. (See Section 2, Article VII, of the Constitution, published in *Anat. Record*, Vol. 4.)

President Harrison announced that he had appointed J. Playfair McMurrich to represent this association to meet with representatives from the American Society of Zoölogists and the American Society of Naturalists for the purpose of formulating plans for the federation of these organizations with a view of obtaining coördination at the annual meetings.

The question of publishing abstracts of papers presented at the meetings was discussed by Knowler, Huntington, McMurrich, Huber and others. The following motion, presented by George S. Huntington, was seconded and carried: Moved that beginning with the next annual meeting members intending to present papers at such meeting be required to furnish the Secretary with an abstract for publication in the Proceedings of the Association at the time of sending in the titles for inclusion in the official program of the meeting.

Henry McE. Knowler moved that a committee of three be appointed from this Association for the purpose of standardizing the courses in biology required in premedical courses and leading to the study of anatomy. Motion seconded by E. A. Spitzka, and carried. The President later announced as such Committee, Henry McE. Knowler, Chairman; Frederic T. Lewis, Warren H. Lewis. On motion the business meeting adjourned.

At the conclusion of the scientific program on Wednesday the following business was presented:

G. Carl Huber proposed the following amendment of Article VI of the constitution: The first sentence of the article "The annual dues shall be \$5.00"—it is proposed to amend to read "The annual dues shall be \$7.00." This becomes a matter of record and will be acted upon at the next annual meeting (see Section 2, Article VII of the Constitution).



On motion the Association tendered its sincere thanks and appreciation to Professor Piersol and the members of his staff, and the local committee, and to Provost Smith and other officials of the University of Pennsylvania for the very efficient arrangements made and for their hearty coöperation in furthering the success of this meeting.

G. CARL HUBER,

Secretary-Treasurer of the Thirtieth Session  
of the American Association of Anatomists

The following Scientific Program was presented and is here recorded by abstracts or titles.

MONDAY, DECEMBER 29, 10.30 A.M. TO 12.30 P.M., PRESIDENT  
ROSS G. HARRISON, PRESIDING.

1. *The development of the lymphatic system in the trout.*<sup>1</sup> CHARLES F. W.  
McCLURE, Princeton University.

My investigations on the development of the lymphatic system in fishes have been confined to the vessels of the head and pharynx. The fishes thus far studied include *Amia calva*, *Lepidosteus osseus*, *Salmo Gairdneri* (steelhead trout), *Salmo irideus* (rainbow trout) and *Salvelinus fontinalis* (brook trout). The vascular system of between 600 and 700 trout embryos has been injected and embryos studied both in transparent mounts and in sections. Forty-two reconstructions after the method of Born have also been made of the arteries, veins and lymphatics in the head and pharynx regions of *Amia*, *Lepidosteus* and the trout, which illustrate the development of the lymphatic system from the time of first appearance, up to the establishment of a condition, in which a continuous system of channels is present. Since the injection method has been employed in following the development of the lymphatics in the trout, I will confine my remarks, for the most part, to the conditions met with in this form.

The injection method shows that the channel system (lumina) of the developing lymphatics, is not continuous at its inception with that of the veins, but is represented by a series of independent and discontinuous lymph sacs or lymph spaces, which subsequently become confluent with one another and, at definite points, join with the veins, to form the continuous lymphatic system of the adult. The principle involved in the development of the lymphatic system therefore appears to be essentially the same as that met with in the yolk blastoderm of vertebrates, where the continuous system of lumina of the blood-vascular plexus, is formed

<sup>1</sup> Read before Section I, of the Seventeenth International Congress of Medicine held in London in 1913.

through the confluence of independent and discontinuous vascular spaces and the endothelium which lines these spaces, is formed from cells which possess a local origin.

At the time when a continuous system of lymphatics is *first* met with in the trout embryo, they are represented, on each side of the body, by the following main vessels:

1. *The lateral pharyngeal lymphatic.* This vessel occupies a superficial position in the lateral wall of the pharynx and forms the direct anterior continuation in this region of the *lymphatic of the lateral line of trunk*. The lateral pharyngeal lymphatic may communicate with the precardinal vein at the cardino-Cuvierian junction, in common with the lymphatic of the lateral line of the trunk; with the precardinal vein near the caudal end of the otocyst or at both of these points. These points of communication with the veins may be single or multiple in character.

2. *The subocular lymph sac.* This is a relatively huge lymph sac at this stage of development, which lies ventro-medial to the caudal half of each eye and drains into the veins, only through the lateral pharyngeal lymphatic, at the typical points of entry mentioned above.

3. *The medial pharyngeal lymphatic.* This vessel lies medial to and is more deeply situated than the lateral pharyngeal lymphatic. It runs an oblique course, in a postero-anterior direction, from about the middle of the lateral pharyngeal lymphatic, with which it often communicates, to open into the precardinal vein just caudad of the point where the latter leaves the cranial cavity.

4. *The precardinal or jugular lymphatics.* These vessels develop along the line of the precardinal veins and drain into the lateral pharyngeal lymphatic near the caudal end of the otocyst. In later stages the *mesenteric lymphatics* drain into the lateral pharyngeal lymphatic through this system of vessels.

At such a stage of development as that just described, all of the above-mentioned lymphatics, including the subocular lymph sacs, can be readily injected from the veins. Also, at this time, blood can and often does pass from the veins into the lymphatics. In one special case, blood was observed to pass from the precardinal vein into the left lateral pharyngeal lymphatic of a living trout embryo and, after completely filling the left subocular lymph sac, to flow back almost immediately into the vein. The passage of blood from the veins into the lymphatics ceases after the veno-lymphatic valves have been formed and observations made upon the living trout embryo, lead me to believe that the passage of blood into the lymphatics, before the valves are formed, is not of any functional significance in the economy of the vascular system, but is due rather to certain local hydrostatic conditions, possibly related to the intermittent flow of the lymph into the veins, as well as to the handling of the embryo under observation. Whatever the case may be, I am convinced that the lymphatics of the trout embryo are not transformed veins.

The subocular lymph sac *can be observed in the living trout embryo* almost from the time of its first appearance and, on account of the relatively large size it attains, is not paralleled by any other lymph structure,

I know of, for convenience of observation and experiment. On account of its large size, the development of the subocular lymph sac is best followed in the steelhead trout (*salmo Gairdneri*), where it makes its first appearance in the embryo between the thirteenth and sixteenth days, depending upon the temperature of the water in which the embryos have been hatched. As far as I have been able to observe in the material at hand, the subocular lymph sac makes its first appearance in the form of spaces or clefts in the mesenchyme which occur medial to the caudal end of the eye. These spaces finally become confluent to form at first a multilocular and then a single-chambered sac. For a period of from 5 to 7 days after its first appearance in the embryo, *each subocular lymph sac serves as a local and independent reservoir for the reception and retention of lymph which it obtains from the head-region and which it retains, until the sac makes a connection with the lateral pharyngeal lymphatic, through which it then drains into the veins. Prior to the establishment of this connection, I have been unable to inject the subocular lymph sacs through the veins or the veins through the subocular sacs.*

Subocular lymph sacs are also found in the embryos of ganoids, but differ from those in the trout in that they drain directly into the veins, in an unmistakable manner, during a very limited period of embryonic development. They then become detached from the veins (12 mm. *Amia* and 14 mm. *Lepidosteus*) and, as far as I have been able to determine without the aid of injections, remain detached from the veins, as well as from the rest of the lymphatic system, even in embryos of *Amia* which have attained a size of 40 mm. in length. The area drained by the subocular lymph sac in the trout, appears to be drained in the ganoids by a lymphatic, not present in the trout, which opens into the anterior end of the lateral pharyngeal lymphatic. Whether the subocular lymph sacs of ganoids, like the caudal lymph hearts of some birds, are only evanescent structures which are not carried into the adult, I am unable to state at the present writing. In consideration of the supposed relationship which exists between the teleosts and the ganoids, it would not be surprising to find a stage of development in which the subocular lymph sac of the trout, like that of the ganoids, drained temporarily into the veins. Such a stage, however, I have thus far been unable to find in any of my injected trout embryos.

Coincident with the development and growth of the subocular lymph sacs in the trout, discontinuous and independent lymph sacs or spaces are being formed, along the lines subsequently followed by the other main lymph channels. These spaces or sacs never approach in size that of the subocular lymph sacs, but like the latter, appear to serve as independent and temporary reservoirs for the reception of lymph prior to the establishment of a communication between their lumina and that of the veins. Those lymph sacs which lie contiguous to the caudal end of the otocyst (otic lymph sac) and to the cardinal-Cuvierian junction (cardino-Cuvierian lymph sac), may establish a communication with the veins, which is practically coincident with their first appearance in the mesenchyme and they are then capable of being injected.



Those independent lymph sacs, however, which lie remote from these sacs or remote from the points at which permanent communications are established with the veins, cannot be injected from the veins, until after they have become confluent with lymph sacs which lie opposite to and communicate with the veins, at the specified points of communication which, as far as I know, are retained in the adult.

Ultimately, all of the discontinuous lymph spaces or sacs become confluent to form a continuous system of vessels. The rate, however, at which this confluence takes place is extremely variable, not only among different embryos of the same age, but even upon opposite sides of the same embryo. In one series of steelhead trout embryos, hatched at a temperature of about 10.5°C., both subocular lymph sacs, in the majority of the embryos examined, had established a connection with the lateral pharyngeal lymphatic on the twenty second day after fertilization, and could be readily injected from the veins. In some of the embryos of this same series, however, this connection had been established on one side of the body only and the lateral pharyngeal lymphatic of the opposite side, extended and could be injected to a point near the subocular lymph sac, but did not connect with the same. Injection experiments have proved conclusively that the subocular lymph sacs do not grow caudad. It is through a centripetal confluence of the lymph sacs or lymph spaces which lie in the course followed by the lateral pharyngeal lymphatic, that the latter vessel is formed, before its connection with the subocular lymph sac is established.

2. *The genetic relations of lymphatic and haemal vascular channels in the embryos of Amniotes.* GEO. S. HUNTINGTON, Columbia University, New York.

In certain regions in amniote embryos lymphatic vessels develop, during the early stages, primarily for the purpose of conveying red blood cells formed *in situ* in the adjacent haemopoietic mesenchyme directly into the venous channels. Functionally these early lymphatic vessels are essentially *haemophoric*. During the period of this functional activity they offer no morphological criteria differentiating them from the adjacent haemal channels. Much of the confusion of terms and of interpretation found in the records of the recent investigations into the development of the lymphatic system is due to the misconception of the early functional character of these primitive lymphatics. They have, owing to their blood cell contents, been classed indiscriminately as venous tributaries or venous derivatives. In the course of further development these early haemophoric lymphatics may, after performing their primitive function, atrophy completely and disappear as components of the definite lymphatic system, as in the case of the proximal portion of the primitive ulnar lymphatic of the mammal. In other regions the early haemophoric lymphatics, after conveying the developing blood cells to their destination within the lumen of the large veins, are retained as functional lymphatic components. The jugular lymph-sacs (anterior lymph hearts) of mammalian, avian and reptilian em-



bryos are examples of this condition. Also, as recently discovered by Miller, the avian thoracic duct. The development of the systemic lymphatics in embryos of the three amniote classes can be compared in the region of the main axial channels (thoracic ducts).

1. In the reptile (chelonian and lacertilia) the large adult periaortal lymphatic sinuses develop at first as small intercellular clefts in the spongy mesenchyme surrounding the dorsal aortic arches and the median dorsal aorta. These spaces enlarge, approach each other, fuse and finally surround the aorta as a huge periarterial lymphatic sinus, with trabeculae in the interior, representing remnants of the original mesenchymal partitions between the components of the sac. This extensive periaortal lymphatic sinus of the reptiles represents the much reduced thoracic ducts of birds and mammals. It establishes secondary connections with the independently developed peripheral lymphatic channels, joins the jugular lymph sacs, and through them attains its entry into the venous system.

From its earliest inception in intercellular mesenchymal spaces the reptilian periaortic sinus is at no point in relation to the venous system. It is closely applied to the dorsal aorta, but there are, at the site of its development, no large embryonal venous channels corresponding to the mammalian azygos (post- and supracardinal) trunks. Consequently the developing thoracic, or rather coelomic, lymphatic sinuses of the reptile never come into intimate genetic or topographical relations with axial veins.

Further, the axial periaortic mesenchyme of the reptilian embryo is not the site of an active intraembryonic haemopoiesis. Consequently, in strong contrast with the avian type, the reptilian homologues of the thoracic ducts never become *haemophoric*.

2. The bird follows the general reptilian type of development, with the following important modifications:

The periaortal mesenchyme of the chick is the site of a most active and abundant intraembryonic haemopoiesis. Masses of developing blood-cells differentiate as axial strands, the "mesenchymal chords" of Sala (1900), ventral to the aorta, directly from the indifferent periaortic mesenchymal syncytium. Subsequently the anlagen of the thoracic ducts appear in this periaortic area as isolated intercellular mesenchymal clefts and spaces. These spaces become confluent, receive the blood cells developed in the periaxial blood islands, convey them through the channels of the thoracic ducts to the jugular lymph sacs, and through them into the circulating venous stream. After this evacuation of their early blood contents the axial lymphatic channels are retained as the permanent avian thoracic ducts (Miller).

3. In the mammal, as shown by a number of recent investigations, the anlagen of the thoracic ducts develop as independent intercellular mesenchymal spaces surrounding the temporary ventro-medial tributary plexus of the azygos veins. Subsequently these venous radicles, enveloped by the growing lymphatic spaces, become detached from the azygos veins, atrophy, and are finally *replaced topographically* by the

mammalian thoracic ducts. This type of lymphatic development has been described by McClure and myself as the "extra-intimal," because the lumen of the lymphatic anlage is always *ectal* of the intimal lining of the degenerating vein, which the resulting lymphatic channel is destined to replace.

Hence each amniote class offers special and peculiar developmental conditions in this particular region. Differing at the first glance widely from each other, they all conform to a common genetic ground-plan, if the same is interpreted in terms of the relation of the first lymphatic anlage to the early periaxial development of blood cells. The reptilian embryo offers in this region the clearest and least complicated illustration of the basic principle underlying all vertebrate vasculogenesis in general and all vertebrate lymphatic development in particular, namely, the formation of a system of connected channels, developed by fusion of originally separate and independent intercellular mesenchymal spaces not complicated by any relation whatsoever to the systemic veins, nor charged with the *haemophoric* function of conveying red blood cells developed *in situ* into the general haemal circulation.

In the bird the periaortic mesenchymal spaces and the resulting channels of the periaortic (thoracic) lymphatic ducts become in the early development stages charged with the duty of conveying the products of the active periaortic mesenchymal haemopoiesis of the bird, as free red blood cells, into the general haemal circulation. Hence, in the bird, we must recognize a distinct *haemophoric* stage in the ontogeny of the axial (thoracic duct) lymphatic channel. In the mammal, the products of an early haemopoiesis of the periaortic mesenchyme are conveyed directly into the blood vascular system through the ventro-medial tributaries of the azygos (supra-cardinal) axial veins. These tributaries having performed this function, atrophy and are replaced topographically by the anlagen of the thoracic ducts, which develop as independent intercellular mesenchymal clefts, surrounding the degenerating venous radicles as the "extra intimal" lymphatic anlagen described in detail by Huntington and McClure. These mammalian "extra-intimal" lymphatic anlagen finally replace altogether the early haemophoric ventro-medial azygos venous plexus, unite with each other to form the channel of the thoracic ducts and make their secondary centripetal connection with the venous system through the link of the jugular lymph sacs. In all three classes of amniote embryos the final result of the genetic processes above outlined is identical, namely, the establishment of a periaortic or paraaortic lymphatic channel, the *amniote thoracic duct*.

3. *Early stages of vasculogenesis in the cat (Felis domestica) with especial reference to the mesenchymal origin of endothelium.* H. VON W. SCHULTE.

From the Anatomical Laboratory of Columbia University.

The variety of the products of endothelium-mesenchyme (v. Szity, Huntington), connective tissue (Boll, Mall) and blood-cells (Maximow, Dantschakoff, Weidenreich, Mollier) would seem decisively to invalidate the doctrine of the specificity of endothelium advanced by Sabin and



other American investigators, and to establish beyond peradventure the close affinities of endothelium and mesenchyme. But if these facts are duly recognized, there is no logical ground for attributing to endothelium a peculiar origin (preferably entodermal), and mode of increase (solely by homoplastic proliferation), or an early and complete independence of the mesoderm, still less of going to the extreme of assigning to endothelium and blood the value of a fourth germ layer, the angioblast of His and Minot.

In the splanchnopleure, in which the early phases of vasculogenesis have been chiefly studied, observation is rendered somewhat difficult on account of the precocity and extent of the vascular anlagen and blood islands, which seem to have caused the scanty but ever present mesenchyme to be overlooked, so that the *Gefäßfaserblatt* has come to be simply the *Gefäßblatt* of many recent observers.

The somatopleure is a more favorable site for the study of the early phases of vasculogenesis, because the process is less rapid, the vascular anlagen do not preponderate and mask the presence of mesenchyme, and it is further widely removed from the entoderm, so that the very remote possibility of an entodermal origin of endothelium is here completely excluded. It may be noted in passing that all investigators of the incipient stages of vasculogenesis in *mammals* are in agreement as to the mesodermal origin of blood and endothelium (Kolliker, Heap, Robinson, Janošik, Bonnet, Fleischman, Keibel, Van der Strich, Maximow, Felix).

Prior to the appearance of the somites, the space between the ectoderm and mesoderm is crossed by fine protoplasmic strands, the fibers of Aurel v. Szity, or interdermal cytodesmata of Studnicka, collectively the mesostroma of the latter author. Along these cytodesmata cells migrate from the mesoderm and form the inception of the mesenchyme. An identical migration occurs even earlier in the splanchnopleure, and in both situations long antedates the resolution of the sclerotomes into mesenchyme. In embryos of two somites and older the migration continues but is reinforced by a separation of cells in groups from ridges of the mesoderm, a process described and figured in the splanchnopleure by Fleischman, with whose results my own are in close agreement. This process may be termed delamination. It is especially active along the lateral margin of the coelom in the position subsequently occupied by the umbilical vein. In some of these masses clefts appear; their enlargement is accompanied by flattening of the enclosing cells; thus separate endothelial vesicles are formed. Similar vesicles are produced, by the same process, above the intermediate cell-masses and have an imperfect segmental arrangement. The intervals between the vesicles are filled with mesenchyme with which their endothelium is in syncytial connection. Some of these mesenchyme cells flatten; at first separated by considerable intervals, the flat cells soon coalesce to form strands and plates; in their protoplasm cleft like lumina appear, enlarge and ultimately coalesce with those of the endothelial vesicles, thus gradually establishing continuous vascular channels. Up to the stage of fourteen somites

the umbilical vein and the associated plexus remain unconnected with the omphalomesenteric vein and the juxta-neural anastomosis.

Identical processes give rise to these vessels also the migration of single elements into the mesostroma, delamination, the formation of discrete vesicles and their ultimate coalescence. Mesenchyme is always present, but scanty in amount.

The first formed vessels of the splanchnopleure are placed in the interval between mesoderm and entoderm. Subsequently they gain more intimate relations with the former layer. From the stage of eight somites they become enclosed between processes of the visceral mesoderm. At the stage of twelve somites and later many of these processes contain funnel-like diverticula of the coelom, the walls of which are intimately united to the blood vessels; the funnels in many instances seem to communicate with the mesenchymal spaces. The presence of these structures, and the further fact that in early stages, just as the first somites are forming, the lateral part of the visceral layer almost wholly resolves itself into mesenchyme, to such a degree that the wall of the coelom becomes incomplete, suggests an intimate morphologic resemblance between the coelom and the tissue space, the further study of which might be expected to throw some light on the general problem of the relations between the coelom and the vascular apparatus as a whole.

4. *On the early contractions of the posterior lymph hearts in chick embryos—their relation to the body movements.* ELEANOR LINTON CLARK AND ELIOT R. CLARK. The Anatomical Department, Johns Hopkins University, Baltimore.

Living chick embryos were observed in a warm chamber, under the binocular microscope. Violent movements, involving the whole musculature of the embryo, were observed at all stages, from four days to the time of hatching. These movements were found to occur periodically: definite periodic spasms of bodily contractions were followed by distinct intervals of rest.

Definite pulsations of the posterior lymph heart were observed first in chicks of  $6\frac{1}{2}$  days. The pulsations, at this stage, were found to be intimately connected with the periodic movements of the embryo. In subsequent later stages, the lymph heart gradually becomes independent in its function.

Chicks were kept alive and under continuous observation for from 3 to 5 hours and records kept of each lymph heart beat and of all body movements, in different stages of embryos.

Stage 1. Chicks of  $6\frac{1}{2}$  to 7 days (20–22 mm. before fixation). Here the lymph heart invariably contracted several times during each period of body movements and never in the period of rest between spasms. Moreover, a beat of the lymph heart was always accompanied by a movement of the tail. When an embryo of this stage was anaesthetized with chlorotone, both body movements and lymph heart contractions ceased at the same time. When the effect of the chlorotone wore off,



the periodic spasms and lymph heart pulsations returned simultaneously and continued as before.

Stage 2. 7 to 7½ days (22–24 mm.). The same as stage 1 except that occasional beats of the lymph heart were dissociated from movements of the tail.

Stage 3. 8 days—24½ mm. The lymph heart contracted several times during each periodic spasm of body movements and occasionally it contracted once, independently, during the period of rest. When the body movements were paralyzed by chloretone, the lymph heart pulsations continued. They did not occur in periodic groups, however, as before the addition of chloretone, but singly, at irregular intervals, from 4 to 8 times every minute. With the return of the body movements, the lymph heart was again observed to contract several times during each periodic spasm, but it also continued to beat, independently, several times in each period of rest.

Stage 4. 8½ to 9 days (27–29 mm.). Fewer beats of the lymph heart occurred during the periodic spasms, than in earlier stages, and more in the period of rest. When the body movements were eliminated by means of chloretone anaesthesia, the lymph heart beat, independently, at irregular intervals,—about 6 to 8 times per minute.

Stage 5. Finally, in a chick of 11 days, the lymph heart pulsations were entirely independent of the periodic bodily movements. Beats were seen to occur during the periodic spasms, but the intervals between such beats were not shorter than between those occurring in the periods of rest, and we observed several spasms during which no lymph heart pulsations occurred.

We have studied, in cross sections, the same embryos observed in the living but we are unable, at present, to offer any conclusive anatomical explanation for the intimate connection between the early pulsations of the lymph heart and the periodic movements of the embryo, and for the gradual manner in which the lymph heart becomes entirely independent.

5. *On certain morphological and staining characteristics of the nuclei of lymphatic and blood-vascular endothelium and of mesenchyme cells, in chick embryos.* ELIOT R. CLARK, The Anatomical Department, Johns Hopkins University, Baltimore.

The nuclei of lymphatic and blood capillaries in chick embryos possess morphological and staining characteristics which differentiate them from the nuclei of the surrounding mesenchyme cells. These differences were noted in cross sections of chick embryos in which the blood-vessels were completely injected with india ink, which were fixed in Helly's fluid, carefully dehydrated, imbedded in paraffin, sectioned, and stained with Ehrlich's hematoxylin and eosin orange G and aurantia; Embryos of from 4¾ to 8 days of incubation were studied.

The nuclei of lymphatic and blood-vessel endothelium have either a single nucleolus or a pair of nucleoli which are definite discoid bodies, sharply marked out from the remainder of the nuclear material, with clear-cut, rounded outlines. The single nucleolus varies much in shape,

according to the shape of the nucleus. These nucleoli have a distinctly reddish color. The remainder of the nucleus has a rather pale, fairly homogeneous granular appearance. The nucleus of the blood-capillary is slightly smaller than that of the lymphatic and nearly always has two nucleoli. The single nucleolus appears to be more common in the nuclei of new-forming sprouts. Frequent mitotic figures may be seen in the endothelium of early lymphatics.

The nucleus of the mesenchyme cell differs in all the particulars mentioned. It contains two or more nucleoli which are *not* sharply differentiated from the remainder of the chromatin material of the nucleus, but which extend out into prongs and threads, and it does not have a characteristic shape. It stains distinctly bluish. The remainder of the nucleus has a slightly darker appearance and often has small clumps of chromatin material.

The fact that the nucleus of the lymphatic endothelium possesses distinct morphological characteristics different from those of the mesenchyme cells, in chick embryos, and that these characteristics are present in the earliest recognizable stages of lymphatic development, coupled with the fact that the nuclei of blood-vessels, with which the lymphatics are connected, have quite similar characteristics, furnishes a new proof that the lymphatic endothelium is derived from the veins. It also assists the study in serial sections of the earliest lymphatics.

6. *The development of the azygos veins as shown in injected pig embryos.*

FLORENCE R. SABIN, Anatomical Laboratory, Johns Hopkins University.

In studying a subject which has been so extensively investigated as that of the development of the veins, it is necessary to attack the problem by some improved method. The method I have employed consists in making dissections or in studying total specimens of injected pig embryos which have been cleared by the Spalteholz method. The first specimens used were the complete injections of India ink made through the umbilical artery by the method developed in this laboratory. Subsequently injections of 0.25 per cent silver nitrate have proved a great step in advance for they enable one to obtain pure arterial or pure venous injections. Pure venous injections for example are a great aid in unraveling the circulation of an organ like the Wolffian bodies. The method of Spalteholz for clearing preparations is well known from his article "Ueber das Durchsichtigmachen von menschlichen und tierischen Präparaten," published by S. Hirzel, Leipzig, 1911. For embryonic material I use 10 per cent hydrogen peroxide for the bleaching instead of the full strength. The bleaching and the thorough washing are important parts of the Spalteholz technique.

A study of the azygos veins must be based on a study of the veins of the Wolffian bodies. Starting with embryos 7 to 8 mm. long, a stage in which the posterior cardinal veins have already been incorporated into the Wolffian bodies, the main veins of the organ are three longitudinal surface vessels, a dorsal or the posterior cardinal vein, a ventral or the



ventro-lateral vein and a mesial or the subcardinal vein of F. T. Lewis. The dorsal vein extends along the dorsal border and receives the segmental spinal veins. The ventral vein extends in the ridge in which lies the Wolffian duct and which marks a general boundary between a mesial glomerular zone and a lateral tubular zone as seen from the ventral aspect. The vein lies mesial to the duct. The dorsal and ventral veins join at the anterior pole of the Wolffian bodies. The subcardinal vein runs obliquely along the mesial surface of the Wolffian bodies. It does not join the posterior cardinal vein at the anterior pole of the organ but rather at a short distance from the anterior pole. It lies ventral to the mesonephritic arteries in the angle between the Wolffian bodies and the root of the mesentery in the position described by Lewis. At the lower pole of the organ it anastomoses with the ventro-lateral vein. Opposite the middle of the organ is the large anastomosis between the subcardinal veins of the two sides making the mesonephritic vein of Minot. The right subcardinal differs from the left, as Lewis discovered in that its anterior end is continued forward into the caval mesentery to the liver making the vena cava. The subcardinal veins are essentially the mesial veins of the Wolffian bodies, for only on the right side a short trunk of the veins which makes the anastomosis with the liver sinusoids lies outside of the organ within the caval mesentery. In embryo pigs 7 to 8 mm. long the mesial longitudinal vein is the largest of the three veins.

Besides these three longitudinal veins there is a long series of parallel veins transverse to the longitudinal axis of the organ which run just beneath the capsule and connect the three longitudinal veins. It is these transverse veins which eventually become the main veins of the Wolffian bodies, that is the main roots of the vena cava. As seen from the lateral aspect, the transverse veins connecting the dorsal and ventral veins are small, of about uniform size and very numerous. In injected specimens they give a ladder like effect to the lateral surface. They run parallel to the tubules. On the mesial surface in embryos 7 to 8 mm. long the transverse veins at the anterior pole cephalic to the mesial vein are very small. The first large transverse vein is the connection of the mesial vein with the posterior cardinal. From this point caudalward there is a series of very large transverse veins crossing the dorso-mesial surface of the Wolffian bodies and connecting the subcardinal vein with the posterior cardinal. They pass ventral to the mesonephritic arteries. The largest of them is opposite the middle of the organ where the two subcardinals anastomose, indeed the mesonephritic vein might as well be called an anastomosis of the two middle transverse veins. In embryos 7 to 8 mm. long most of the blood from the mesial part of the Wolffian bodies passes by the subcardinal trunks through the transverse veins to the posterior cardinal veins and the anastomosis between the right subcardinal and the liver is small. When the embryo is 11 to 12 mm. long the anastomosis with the liver is large and the subcardinal veins are the main roots of the vena cava. By the time the pig is 15 mm. long the vena cava has become very large and the middle transverse veins are its largest roots in the Wolffian bodies, while the posterior cardinal and

ventro-lateral veins have become limited to the anterior pole of the organ. It is at this point that the azygos veins begin.

There have been two theories concerning the origin of the azygos veins, the more accepted one that of Hochstetter that the azygos veins are at least in part transformed posterior cardinal veins: the other advanced by Parker and Tozier from the Harvard laboratory in 1897 and in the same year by Zumstein that the azygos veins are new veins. That this latter view is the correct one I can prove by dissections of injected embryos showing the two veins in the same specimen. In stages below 14 mm. the spinal veins pass directly to the Wolffian bodies in a straight line parallel to the mesial sagittal plane from the spinal ganglia and the tissue dorsal to the aorta around the notochord is non-vascular. At the stage of 14 mm. there develops from the spinal arteries a capillary plexus, ventral to the vertebrae. These capillaries begin in the cervical region and drain by many branches into the anterior cardinal vein and lower down into the posterior cardinal. In the body region a longitudinal vein develops in this plexus which retains as its permanent connections with the cardinal veins the branches which join the posterior cardinal vein at the point where it curves ventralward to make the duct of Cuvier. This point of connection, as is well known, is at first high up at the root of the neck and gradually shifts caudalward. The only part of the azygos system which is derived from the cardinal system is the ventral curve of the duct of Cuvier. The permanent pattern of the veins in the pig is as follows: on the left side a hemiazygos and an accessory hemiazygos enter the heart through a permanent duct of Cuvier, on the right side the azygos vein joins the cardinal at the same level as on the left side but the duct of Cuvier is longer. Corresponding to the accessory hemiazygos there is a larger oblique vein draining more than half of the prevertebral tissue of the first four vertebrae which was described by Kampmeier as a vein which disappears as the thoracic duct develops. Injections show that it is a developing vein at the time when Kampmeier thought it disappearing. Injections of embryo pigs from 20 to 25 mm. long show the complete posterior cardinal veins together with the azygos and hemiazygos systems. The posterior cardinal vein is always farther ventral and farther lateral than the azygos. The azygos veins are dorso-lateral to the aorta. Eventually the posterior cardinal veins become tributaries of the azygos system.

7. *A comparative study of the embryonic blood vessels and lymphatics in amphibia.* HENRY McE. KNOWER, University of Cincinnati

In order to understand the development of the lymphatic system, it was necessary first to secure accurate knowledge of the primary arteries and veins and their capillary beds, in relation to regions and organ rudiments, at different stages of the early development of the forms studied. It then became possible to make comparisons within and outside of the group; and to examine and discuss safely the lymphatic system.

Hence this paper is naturally divided, on the one hand, into a section devoted to an outline of the results of a study of the primary vascular



system of amphibia; with its origin, most important relations, and transformations, as well as a comparative study of these problems and the origin of the blood; while, on the other hand, the second section is concerned with the development of lymphatics in amphibia; the relation of this system to other systems of the body, especially to the tissue spaces and pronephros and mesonephros; the development of lymph hearts; as well as with a comparative discussion of these findings, involving a comprehensive working hypothesis of physiological and experimental nature for the development of the lymphatic system in vertebrates.

The elaboration of proof of so extensive a program will, of course, demand much more space than is here available.

The method of investigation is predominantly experimental and involves a study of each embryo as a whole. Injections were used not simply to secure a series of morphological forms for comparison, but rather to exhibit and fix for study relations of physiological balance between the various vascular beds (dorsal, ventral, lateral, antero-posterior) and the regions of the body, at different critical periods of the embryo's history. It is thus possible to show how, especially in the formative stages, pathways will be opened along lines determined by usual or extraordinary balances in pressure relation; and how in agreement with Mall (on the liver) and Thoma, Evans and Sterzi, and so forth, yet with additions, the main vessels are established in the amphibian embryo as a result of the fixation of certain physiological streams flowing more and more constantly through the capillary anastomoses of different regions.

Young amphibian embryos are especially favorable for such study. The simplicity of the entire organism, which can be cleared and viewed in one field; its availability for observation and experiment while alive; and the important relationships to other forms which permit us to apply our studies to general problems; have, we believe, furnished us a special insight into the problems involved. This carries us some steps further, because it has been possible in the study of the system selected (that is, the lymphatic system) to keep more constantly in touch with the stages of the other systems of the body, whether arteries, veins, organs or tissues, as parts of one organic mechanism. The interaction of the parts as affecting the problem of development of the vessels and lymphatics has been tested by experimental injections, and otherwise.

Hoyer for the amphibia, and others for other groups, have made most important contributions by studying one system at a time, more or less as an entity, either by models or injections. They have aimed to arrive at morphological comparisons and genetic relations of the system.

It is not to be denied that many observations of living embryos, models, and injections, in our sense, have advanced our knowledge. Evans has brought this together in a most able manner ('13). These methods have not, however, proved entirely adequate for reaching an appreciation of some very important problems of inter-dependence of all systems as affecting lymphatics; nor for grasping some essentials in

the development of blood vessels and lymphatics which might prove common to all methods of approach.

Another great advantage in our studies has been the fact that many of the injections, by our special method, were of far earlier stages of both Urodeles and Anura than have been secured by others. This has enabled us to clear up some points in the establishment of primary vessels not otherwise possible.

Our previous work, experimental and other, has been confirmed and extended.

I. Successful injections of young *Amblystoma* embryos before the post-cardinals become defined, furnish a variety of specimens in the same or closely related stages, depending upon the physiological state of the embryo. It can be shown that the blood from the dorsal aorta leaves this vessel, behind the point where it begins, ventro-laterally all along its course, on either side. We do not find the extremely large sheels nor the same history as von Mollendorf, but can agree with some of his points. Each lateral stream branches into two, a dorsal and a latero-ventral. The latter vessels run ventrally over the yolk in a fairly wide plexus.

In the next stages the blood returning from the bifurcated caudal ends of the aorta, tends to fix a pathway from behind to the pronephric sinus through the vitelline plexus, on either side, along and under the edges of the myotomes. This return stream to the pronephric sinus will become the postcardinal. It flows through the vitelline anastomosis, and tends to push forward over these outcoming streams, so that when later the vitelline arteries lose connections with the postcardinals, they are found beneath (ventral to) these. There is then, for a time, a free connection from the aorta to the forming postcardinals and outward to the vitelline plexus. The dorsal or neural arteries run up from near the division of the latero-ventrals into their postcardinal (lateral) and vitelline (ventral) branches.

Anteriorly, in the region of the pronephric glomerulus, practically the same condition as behind is found. Several pronephric arteries are found running laterally from the aorta, in intimate association with the vitellines of this region related to the vitellines as the lateral vessels to the postcardinals are related to the vitellines in the posterior part of the body. The glomerulus is not a mere saccular enlargement as formerly described, but rather a plexus. It has a venous drainage.

The posterior cardinal is thus a fixation and separation of a venous return from lateral branches of the aorta. These branches are at first also in connection with the neural plexus through the dorsals, along the sides of the aorta. Hence the dorsal, lateral, and ventral aortic branches are to be regarded as primarily derived from one series of latero-ventrals which branch in three directions. Variations as found by Goeppert are numerous in the origin of laterals and ventrals from the aorta.

In frog embryos the postcardinal loses its connection with the sides of the aorta at a very early stage, except in front and behind. Birds also exhibit a secondary condition in this respect, since Evans found no



aortic connections in the mid-body. The anterior and posterior regions remain more primitive. My experimental work of 1907 shows that in frogs the primary connections of aorta and cardinals can be forced to persist.

The umbilical artery of higher forms, as well as the limb-plexus arises from lateral loops of the dorso-lateral aortic branches which are connected, as Hochstetter and Evans claim, from the beginning in the primitive manner just indicated, with the postcardinal and splanchnic vitelline plexus. There is a fundamentally similar condition in amphibia, well shown in young *Necturus* embryos.

The formation of the definitive arteries and veins of the various divisions of the digestive tract, as esophagus, stomach, liver, intestine, and hind-gut, have been studied. They arise as transformations from plexuses, secondarily, as a result of changes and movements in the tissues and organs of the regions concerned. This is in agreement fundamentally with Mall, Evans, Bremer and in many features with von Mollendorf, etc., for the establishment of the larger trunks in other forms.

These studies go to prove a fundamental similarity between the primary vessels and plexuses of amphibia and those of other vertebrates. We find no 'essential' difference between the vascular systems of anamniotes on which to base such distinctions as have recently been drawn by Elze. Differences are of degree rather than kind, and we regret that we cannot subscribe to a number of Elze's claims.

Elze's use of our experiments to support his contention is entirely unwarranted; for although these frog embryos lived two weeks without a heart, they grew abnormal as they became more and more dependent upon skin breathing alone, in the absence of normal circulation; and, I should now add, in the absence also of a normal excretory apparatus.

There seem to be important bearings of our findings, taken in connection with the results of others and with our own experimental work, on the questions involved in the establishment of the angioblast and first vessels; the extension of these in the body and over the yolk; and the origin of blood cells.

On the whole, we agree with Bremer ('12) as to the almost simultaneous origin of aortae, early gill arches, and the vitelline foundations of the postcardinals. We should modify von Mollendorf's results somewhat for our forms. The angioblast appears to us to consist first of an anastomosing mesh, including heart, early gill loops with the anterior part of the aorta and a venous return to the heart through simple vitelline loops, lying dorso-laterally on the yolk. This mesh is fairly continuous. It progresses backward on either side of the aorta as the terminal loops of the aorta push back. In this way vaso-formative cells and hematopoietic cells are established as claimed by many authors, on either side, extending back from the heart, along the line of the aorta, and of the origins of the vitelline arteries to the base of the forming tail. As the embryo grows longer, the movement of the vascular rudiment is a general one, in one system of anastomosing loops, backward along the

dorso-lateral aspect of the yolk and further out into the tail. It does not seem important for all parts of the extensive rudiment to exhibit a lumen at once. The loops are influenced by tissue activities to grow out, while the non-functional formative tips may not everywhere be clearly marked off from surrounding tissues in the early rudiments. Later, the vaso-formative activity of the cells of the rudiment lessens; while the endothelial tubes already formed extend by their own growth in wide plexuses, both backward and ventralward on the yolk and in the body. There is now more difference between the tissue cells and vessel walls.

The writer's personal observations on these questions have not been made on the earliest non-injectible stages; but nevertheless, as he believes, on stages early enough to indicate the nature of the processes, and to show a definite bearing on his experiments of 1907.

Since the line of extension of the angioblast lies along the roots of the vitelline arteries, that is, also the roots of the mesentery, as far as the base of the tail, it is significant that embryos from which the heart is removed at an early stage ('07) later exhibit collections of blood cells in the mesentery, as well as at the base of the tail.

It must be stated here that a thorough study has also been made of the dorso-lateral, neural, and other blood vessels in order to value properly the conflicting claims in regard to the lymphatics, especially in the region where these first appear.

II. In turning to the lymphatics, we are met by two opposing views: on the one hand, that the lymphatics are outgrowths from the endothelium of the veins; and on the other hand, that they arise by confluence of tissue spaces which run together centripetally to join the veins.

Now, there seems to be no doubt that tissue activities initiate the origin and maintain the lymphatics as a system. But why should tissue spaces collect into relatively large vesicles and run together in such definite lines? Is it proven that such actually become a continuation of the lymphatic system? Why do we not find enlargements at the end of our vessels, representing vesicles from tissue spaces, on injecting lymph capillaries, instead of invariably finding most delicate tips? Why should the main trunks of this system, communicating with the veins as they do, arise separately and by an entirely different method than that followed by the venous trunks, which are returns established through a previously functioning generalized plexus?

The technique is evidently very good on both sides, and in very many points there is possible convergence in interpretation. Both sides proceed on the assumption that nature is constant. Hence, it should not matter whether a stage is studied by models or injections or by combined methods; since there is a closer approximation to the truth in each specimen examined. It should be as possible to interpret the facts with the aid of good injections and sections and other specimens, as by making models to express the same facts and interpretations of a constant stage, similar in many groups. It should be, if anything, easier to determine whether strands of endothelium at the ends of a definite injectable system invade and tap uninjected spaces of connective tissue, than it is to



prove that certain indefinite spaces in the connective tissue combine to build up a system running centripetally in definite lines strictly comparable in various groups.

Can we not accept whatever is proved by either side; and even go further, by associating the development of lymphatics with other systems of the body, and discover a cause in embryos of all vertebrates which will aid us in explaining this system? At least, can we not find a working hypothesis?

Turning to the amphibia, we find it possible to make more definite statements about earlier stages, in both frogs and *amblystoma*, than have been hitherto possible. The first lymph vessels in the frog form a small and superficial dorso-lateral plexus, which drains into the pronephric sinus through a short vein. On this plexus in the frog, the anterior lymph heart soon appears and facilitates the drainage into the venous channels surrounding the pronephric tubules. The Clarks have shown a similar secondary appearance of the posterior lymph hearts after the plexus is formed in birds ('12). The primary lymphatic endothelial plexus may be thought of as being attracted by some chemotaxis, which arises in the tissue spaces as the mesenchyme becomes looser and more vacuolated. This phenomenon of outgrowth is to be observed about the time that the external gills begin to show distinctly, and when the pronephros is organized. The appearance of the first lymphatics at this stage, and in the region of the body where important physiological processes are being inaugurated, suggests strongly that this association is causal. We shall elsewhere give many reasons, and a mass of correlated facts, to justify the view that the early lymphatic plexuses of embryos in all vertebrates are endothelial outgrowths, induced to invade vacuolating tissue spaces by changes in the metabolism of this region. The endothelial lymphatic vessels carry off the accumulated products more directly and rapidly from the tissues than would be possible through tissue spaces. It is our view that they can thus be carried more rapidly to the pronephros for elimination. (See Abel, *Jour. of Pharm.*, 1912).

The lymph hearts appear later at the point of entrance of the plexus into the veins, just adjacent to the pronephros and facilitate the emptying of the plexus into the venous channels surrounding its excretory tubules.

As development proceeds, the changes in the tissues bringing about vacuolization, spaces, and so forth, progress tailward and take place most actively just under the skin and dorso-laterally.

Coincidentally, the lymphatic plexus travels backward, spreading dorsally and ventrally beneath the skin as it moves. The stages are different in important features from those described by Hoyer, whose older stages did not show the true nature of the plexus, though fundamentally we shall be in agreement. In this manner the dorsal and ventral caudal trunks are laid down as the tissues of the tail favor their invasion. A delicate but rather extensively lymphatic plexus comes to overlie the veins at the base of the tail before the appearance of the posterior lymph hearts; (we understand Hoyer to be in agreement with this, though his pupil Fedorowicz seems to disagree).

At first the entire system of lymphatics drains through the anterior lymph hearts into the pronephric sinus. With the inauguration of greater tissue activities in the region of the hind-body, with the development of the limbs and of the Wolffian body with its special venous channels, bathing the tubules, a connection is established between the endothelial tubes of the lymphatics and certain branches of the lateral caudal veins. We think Fedorowicz's observations are incomplete, or on unfavorable material, and that his cell strands in the tissues near the veins before the posterior lymph hearts appear will probably prove to be lymph terminals which have been attracted back, as we find, into this region from in front.

Thus the vacuolated, and, as it were, the oedematous tissue spaces of the posterior portion of the body are now drained through the posterior lymph hearts into the veins of the mesonephros, where an elimination may take place through the tubules which they surround. (These portions of the tubules, as well as the glomerular sections, are claimed by several authorities to be excretory, while the venous streams are passing through the renal-portal system toward the heart).

This opens up some interesting problems of the functions of the different parts of the pronephros and mesonephros as compared in embryos of other forms. The functions of these bodies should also be recompared with those of the kidneys of adult sauropsida and mammals, including man. Since we know of no accurate studies along these lines.

Consistently with these views, the invasion of the viscera by lymphatics from the roots of the mesenteries should follow the developmental activities of these organs in changing from a simple primary tube as the accompanying active histogenesis gives rise to new chemotaxis favoring this. This is true of the development of the thoracic duct in amphibia and in higher forms.

In Urodeles there is essentially the same history; but here an exceptionally extensive lymphatic plexus overlies the veins very closely, and invades the neighboring connective tissue; while the numerous lymph hearts appear later connecting the two systems. Hoyer's recent ('12) figure for late salamander larvae, are not quite reconcilable, but can probably be corrected by study of young and late amblystoma, if he has confused veins with overlying lymphatic vessels in incomplete injections of the trunk region, as seems possible.

Comparisons of these facts and application of this working hypothesis to the embryos of other forms, including man and mammals, where the nature of the pronephros appears to produce interesting variations, will be explained fully elsewhere.

It seems clear that this view of the method and supposed causes which bring about a 'taping' of the enlarging tissue spaces, permits us to use many of the valuable results, not only of the advocate of the importance of the tissues and tissue spaces in the problem, but also of those who are impressed with the continuity of the endothelium as it invades the body.

Extra-intimal spaces may well prove to be lymphatic capillaries which have travelled along the veins and which undoubtedly exist in my specimens.



Though I have not seen convincing cases, demonstrating beyond doubt the opening of lymphatic terminals into tissue spaces, this has been claimed by able observers; and though the necessity for this is not yet shown and the proof must be more final, it will not be inconsistent with my findings and conclusions, if such opening should be shown to be established. Such a condition might facilitate the passage of substances into the endothelial lymphatic vessels when once this plexus had been induced to invade a region.

At any rate, we must now take into account, in the embryos of all vertebrates, the relations of tissue metabolism, respiration, the function and character of lymphatic drainage, with first the pronephros, and later the meso- and meta-nephros. There will be found a remarkable time relation, and correlative association, in both normal, and experimented, and pathological embryos where the function of the kidneys, and so forth, are disturbed. This may lead to a 'dropsy' more or less chronic; which, in certain cases, may even possibly become a habit of a normal stage or species. The jugular sacs of mammals may be somewhat distorted by such influences.

On motion a discussion of the several papers presented at this Session was deferred to the end of the Session and was participated in by George S. Huntington, Henry McE. Knower, Eliot R. Clark, J. Playfair McMurrich, C. F. W. McClure and Charles R. Stockard.

MONDAY, DECEMBER 29, 2.00 P.M. TO 5.00 P.M. SESSION FOR THE READING OF PAPERS, PRESIDENT ROSS G. HARRISON, PRESIDING.

8. *Experiments on the development of blood vessels in the blastoderm of the chick.* ADAM M. MILLER, Anatomical Laboratory, Columbia University, JOHN E. MCWHORTER, Surgical Laboratory, Columbia University.

The object of these experiments on the living blastoderm of the chick has been to derive some evidence bearing on the question of vascularization of the area pellucida and embryonic body. It was assumed that if the entire lateral half of the area opaca was removed from the blastoderm prior to the appearance of vascular anlagen in the area pellucida or embryonic body and the blastoderm was then allowed to proceed in development, it would be possible to test the validity of the view that blood vessels in the area pellucida and embryo proper arise in situ and not as ingrowths or sprouts from antecedent vascular anlagen in the area opaca.

By examination of living blastoderms and serial transverse sections of blastoderms in successive stages of development, it was found that up to the stage in which the 'head process' (primitive axis) was clearly visible on surface view there were no cells between mesoderm and entoderm in the area pellucida or embryonic body.

We sought, therefore, to remove the entire lateral half of the area opaca and the lateral portion of the area pellucida at a stage not later than the complete formation of the primitive streak, and thus to prevent, in the further developing blastoderm, possible ingrowth of vascular anlagen from the area opaca of one side. This accomplished, it would follow that any vessels appearing in the remnant of the area pellucida or in the same side of the embryonic body subsequent to operation must have arisen in situ.

Through a 'window' in the egg shell, and with the aid of a binocular microscope, an incision was made in the blastoderm at the proper stage which effectively separated the area opaca and a portion of the area pellucida on one side from the remainder of the blastoderm. The egg was then further incubated under conditions as nearly approximating the normal as possible. More than 50 blastoderms were operated on and allowed to develop subsequently for periods ranging from 20 to 72 hours.

In general, development went on normally (barring slight retardation) on the uninjured side for at least 24 hours. In some cases the anlage of the heart on this side alone developed. This was probably due to the fact that the incision had been so close to the sagittal mid-plane as to remove the opposite cardiac anlage. Usually after 24 hours the embryo would become abnormal in contour, although the extra-embryonic area continued to develop fairly regularly and the heart continued to beat.

On the injured side, between the sagittal mid-plane and the line of incision, practically all the usual structures developed. The cut edges of ectoderm and entoderm healed together, thereby enclosing the mesoderm. The coelom appeared, although irregular in outline. The somites were found in their usual positions.

Active vasculogenesis was found to occur not only in the remaining portion of the area pellucida but also in the embryonic body. Numerous blood islands of characteristic appearance, as well as vessels destitute of blood cells, developed in the splanchnic mesoderm. The aorta and the cardinal and umbilical veins appeared in proper position. The cells comprising the blood islands were differentiated in loco from the mesoderm (mesenchyme), and the vessels appeared for the most part as series of isolated lacunae, in small part as solid cords which subsequently acquired lumina. In the earlier stages neither the blood islands nor the vessels were connected with vascular structures on the uninjured side.

These results show that the removal of the entire lateral half of the area opaca at a stage prior to the appearance of vascular anlagen in the area pellucida does not prevent subsequent development of blood cells and vessels in the area pellucida or in the embryonic body on the same side of the sagittal mid-plane. It has been found, on the other hand, that after such an injury vascular structures develop in both localities. The conclusion is justifiable that the blood vessels of the area pellucida and embryo do not grow in from an extrinsic region but arise in situ.



Discussed by Knowler and Huntington. In his discussion of this paper Dr. Huntington referred to the fact that Miller and McWhorter's manuscript had been submitted for publication to the Editorial Board of *The American Journal of Anatomy* and had been returned with what seemed to the authors to be irrelevant suggestions for improvement. The chair ruled these remarks as out of order. The chair was overruled. Huntington, Knowler and McMurich participated in the discussion that ensued.

9. *The origin and early development of the posterior lymph heart in the chick.* RANDOLPH WEST, From the Anatomical Laboratories of Princeton and Columbia Universities.

Last year at the suggestion of Professor McClure and under his direction, the writer commenced the investigation of the earliest development of the posterior lymph heart in the chick, and the problem has been continued during the present winter under Dr. Huntington at Columbia University.

As only the early development of the posterior lymph heart has been considered, most of the embryos studied have been between 6.5 and 15 mm. in length, although one or two older ones have also been examined. All of the embryos, with one or two exceptions, were injected with india ink through the viteline vessels, the injection being pushed to the point of extravasation for the haemal capillaries. They were then fixed in Zenker's fluid, sectioned, and stained with eosin and methylblue by Mann's method. A few embryos were preserved entire and cleared by Spalteholz's method.

The posterior lymph heart arises, in the chick, in the mesenchyme lateral to the caudal muscle plate and caudal to the hind limb bud. Before the lymph heart assumes the form of a single sac-like cavity there exists in this same area a plexus of lymphatic vessels, which later coalesce to form the single cavity of the lymph heart. Both the lymphatic plexus and later the lymph heart are in connection with several of the most anterior coecygeal veins by means of their lateral branches which pierce the caudal muscle plate, drain the lymphatics and then pass outward to drain a haemal capillary plexus, which bears a superficial relation to the lymphatic plexus. Concerning the origin and development of this lymphatic plexus two main points have been observed. First, the lymphatic plexus arises by the confluence of independent uninjectible lacunae, bounded at first by indifferent mesenchyme cells which become flattened to form an endothelium. Second, both in the lymphatic endothelium and in the surrounding mesenchyme an active haemopoiesis is taking place. It is also necessary to consider the growth of the superficial haemal capillary plexus in this neighborhood. This haemal capillary plexus extends its borders and becomes richer by the addition of numerous independent blood islands which have been differentiated from the mesenchyme.

It must be remembered that all of the processes alluded to; the space formation, the haemopoiesis, the formation of groups of blood cells to enrich the haemal capillary plexus, take place *only* in the mesenchyme lateral to the caudal muscle plate and caudal to the hind limb bud, and *only* during the short period of embryonic history just prior to and during the formation of the lymph heart.

First, then let us consider the extension of the haemal capillary plexus. In the 6.5 mm. embryo the mesenchyme lateral to the caudal muscle plate is indifferent. In the 7 mm. embryo the lateral branches of the coccygeal veins have pierced the muscle plate and groups of eosinophile cells have appeared in the mesenchyme. In the 8.5 mm. embryo the capillary plexus, drained by the lateral branches of the coccygeal veins is present in the form of a few small vessels, and the groups of eosinophile cells are very abundant. By the time that the embryo has reached the length of 10.5 or 11 mm. the groups of eosinophile cells have practically disappeared and the capillary plexus, now draining the area which they once occupied, has reached a high degree of complexity. So it seems reasonable to conclude that these groups of blood cells have been drained off by the capillary plexus.

The first lymphatic anlagen were observed in the 10.5 mm. embryo. Up to this stage the mesenchyme lateral to the caudal muscle plate has been firm, but now for the first time a distinct loosening of the mesenchyme may be observed near the caudal muscle plate. In the 11 and 12 mm. embryo this space formation becomes more and more pronounced, and some of the spaces have acquired a connection with the lateral branches of the coccygeal veins. In the 13, 14, and 15 mm. embryos the spaces have assumed a comparatively large size and many of those spaces still disconnected with the veins are surrounded by mesenchyme which is becoming flattened to form an endothelium. Of course when these spaces become connected with the veins, the venous blood may back up in them, but this regurgitation of blood from the general circulation is not to be confused with the haemopoiesis which is about to be described.

The formation of groups of blood cells which enrich the haemal capillary plexus has been noted. In addition many mesenchyme cells become rounded and develop into either the white or the red blood cell line as described by Dantschakoff. Many of these blood cells become included in the mesenchymal spaces which form the lymphatic anlagen, and when these spaces join the developing lymphatic plexus, the included blood cells gain access to the general circulation via the lymphatic plexus and the coccygeal veins. Other of the blood cells having the power of amoeboid movement may migrate through the vascular walls, while from the endothelium of the lymphatic plexus a very active haemopoiesis is taking place after the embryos have reached the length of 12 mm.

All the evidence found from the study of injected embryos leads to the conclusion that the lymphatic plexus, which later enters into the formation of the posterior lymph heart, arises by the confluence of independent mesenchymal spaces which connect secondarily with the

veins, that these spaces are bounded by mesenchyme cells which become flattened to form an endothelium, and that both in the endothelial walls and in the adjacent mesenchyme an active haemopoiesis is taking place.

Discussed by Huntington.

10. *Experimental mesothelium.* WILLIAM COGSWELL CLARKE, Department of Surgery, Columbia University.

Following a purely physical injury which destroys the free surface cells of the peritoneum, pleura, or the lining cells of blood vessels, two possibilities exist as to how regeneration of the damaged zone proceeds. (1) cells grow from the periphery of the given denuded area, taking origin from adjacent, previously existing and intact flat surface cells; (2) the exposed deep connective tissue cells making up the floor of the injured area as they proliferate, change in form, becoming flattened.

These experiments were undertaken in reference to the latter possibility in the regeneration of surface cells to learn what happens as regards connective tissue cells in contact with a smooth surface, whether solid or fluid; in other words to learn what change in form takes place in the investing connective tissue cells in contact with the surface of a non-irritating foreign body, placed for a time in the subcutaneous tissue of a living animal.

Non-irritating solid and fluid foreign bodies were used: (1) thin, smooth, chemically clean sterile sheets of celloidin were selected as the best non-irritating foreign body. These foreign bodies were introduced into the living subcutaneous tissue of animals through as small a wound as possible and left for varying periods. Sections were cut both at right angles to and in the plane of the surface cells; (2) paraffin was injected into the cornea, a vessel-free structure, in order to observe later the cells that are found in relation to the surface of the foreign body; (3) those surface cells were also observed which were in relation to stationary collections of fluid exudate in dead spaces or cavities present in the depths of a wound through imperfect coaptation of the walls; (4) finally, in order to introduce the factor of friction as well as of pressure of the given foreign body, a mucous fistula was established. The duct of a dog's gall bladder was obliterated by ligature and a rubber tube was led through the substance of the abdominal wall from the fundus of the gall bladder through the skin of the left flank. At seven days the rubber tube was pulled out and the mucous secreted by the lining epithelium of the gall bladder flowed continuously through the fistula. Since no bile entered the bladder, the fistula carried a nearly bland fluid. A section of the fistula was removed for study at the end of twenty-eight days.

Where possible, sections of all the above specimens were cut in two planes, one at right angles to the lining cells in contact with the surfaces of the above foreign bodies, the other tangential to the surface or lining cells. In the case of the lining cells in contact with celloidin, a silver salt, protargol, was employed to determine the presence or absence of a mosaic similar to that observed in silver preparations of the peritoneal and pleural mesothelium.



The sections showed that the cells in contact with the surface of the foreign bodies were changed in form in all the specimens into large, flat cells placed edge to edge resulting in a definite cellular sheet. The silver salt demonstrated a mosaic of black silvered lines marking the cell outlines of the lining or surface cells. The cells that existed in the mucous fistula were large cells forming at points a continuous lining.

The fact demonstrated in the above experiments that connective tissue cells are changed in form by physical agents into flat, closely disposed cells, the outline of which may be defined by silver salts, makes tenable the conclusion that the exposed connective tissue cells, exposed through sacrifice of surface mesothelial cells of pleura, peritoneum, or pericardium or of the lining endothelial cells of vessels, may become flattened by pressure or friction or both, resulting in regeneration of the surface cells.

Discussed by Huntington, E. R. Clark and Knower.

11. *The behavior of elastic tissue in the postfetal occlusion and ultimate obliteration of certain blood vessels.* J. PARSONS SCHAEFFER, From the Anatomical Laboratory, Department of Medicine, Yale University.

The preliminary paper, "The behavior of elastic tissue in the postfetal occlusion and obliteration of the ductus arteriosus (Botalli) in *Sus scrofa*," on this work appeared in the February number of the *Journal of Experimental Medicine*, vol. 19, 1914, pp. 129-143. Work bearing further on these problems is now in progress, the results of which will be published subsequently.

A brief summary of the paper published in the *Journal of Experimental Medicine* is given herewith:

1. A study of the histogenesis of elastic tissue in the embryonic ductus arteriosus of *Sus scrofa* is in accord with the theory that elastic fibrils are directly differentiated in the outlying portion of the protoplasm of the early connective tissue cell.

2. In the occlusion of the postfetal ductus arteriosus of *Sus scrofa* there is early a hypertrophy of the internal elastic membrane. Subsequently there takes place a marked delamination of the thickened internal elastic membrane in the production of new and independent elastic fibers and lamellae. The formation of new elastic fibers from preformed elastic tissue is most abundant where the postfetal contraction of the ductus arteriosus is least marked. These new elastic fibers play an important part in the occlusion of the lumen of the postfetal ductus.

3. Aside from the extensive formation of elastic fibers from preformed elastic tissue, in the occlusion of the postfetal ductus arteriosus of *Sus scrofa*, there are also some elastic fibrils formed from non-elastic elements, apparently from connective tissue cells.

4. In some recent preliminary work on ligations of the common carotid artery there was found, after an interval of from eight to twelve days, at some points between the ligatures, a slight but obvious cellular thickening of the so-called subendothelial stratum. Some of these cells



may have wandered from the other coats of the vessel, through the inner fenestrated membrane into the subendothelial stratum; others proliferated from cells in loco. Specific stains revealed near the periphery of some of these cells, that is, in the outlying portion of the exoplasm, very delicate, granular-appearing elastic fibrils, apparently the product of protoplasmic activity.

The reader is referred to the original paper for the details of this work.

Discussed by H. M. Evans.

12. *The earliest blood-vessels in man.* J. L. BREMER, Department of Anatomy, Harvard Medical School.

Heretofore it has been generally supposed that in man, as in other vertebrates, the first blood vessels appeared in the yolk-sac, between the entoderm and the mesoderm. The early vascularization of the body-stalk and chorion in man, before the presence of intra-embryonic vessels, and before the formation of somites, has been long noted, but usually considered as evidence of a very rapid growth from the yolk-sac anlagen. In human embryos with the medullary plate of about 1 mm. in length, and with recognizable yolk-sac vessels, several authors have described, in the chorion, chorionic villi, and body-stalk, irregular spaces in the mesoderm, some lined with endothelium, some without definite lining; and recently Grosser and Debeyre have separately mentioned also blood islands in the body-stalk, near the allantois. In still younger embryos, with no vessels or blood islands in the yolk-sac, Jung and later Herzog have called attention to accumulations of cells, occasionally arranged around a lumen, seen here and there at the periphery of the mesoderm of the body-stalk, which Herzog regarded as the earliest anlagen of the yolk-sac blood vessels.

By the recognition of the fact that apparently isolated endothelial spaces, or angiocyts, may be connected by solid cords of endothelium, as demonstrated in a former paper on the origin of the aorta, I was able to reconstruct, in a 1 mm. human embryo in the Harvard Embryological Collection, and in the 1 mm. embryo described in 1913 by Grosser (who most kindly allowed me to study carefully this excellently preserved specimen) continuous nets, composed of angiocyts and solid cords, extending in each embryo throughout the chorion, chorionic villi, and body-stalk. In Grosser's embryo this net anastomoses at one point with the similar net in the yolk-sac; in the other embryo such anastomosis was not found. The 'irregular spaces' thus form part of a vascular system. Young blood corpuscles can occasionally be seen within the angiocyts, and the blood island of Grosser is connected with the net.

Grosser first called special attention to the epithelial layer of mesodermal cells, the mesothelium, which forms the coelomic surface of the yolk-sac and of the body-stalk in his 1 mm. embryo, ending abruptly at the junction of body-stalk and chorion. Moreover he pointed out that this mesothelium, instead of forming a smooth surface, dipped in irregularly, giving in sections the appearance of festoons. I found, in

both embryos, that the prolongations inward, toward the center of the body-stalk, often joined the angiocyts or the cords of the vascular net, and that cells resembling young blood corpuscles occasionally were included in these mesothelial strands. Some of the angiocyts not connected with the general net were seen to be independently connected by such strands with the mesothelium.

In the Herzog embryo, in which there are no blood islands or vessels in the yolk-sac, the mesothelium of the body-stalk is not a complete layer, much of the surface at the edge of the coelom being represented by mesenchymal processes or fibers. Where present, however, the mesothelium occasionally dips inward, thus lining a funnel-shaped extension of the coelom, and in one case at least the end of this funnel can be traced to a solid cord of cells which runs out into the chorion. Other such cords in the chorion can be traced from the body-stalk for some distance, and can be seen to anastomose, forming a net. Herzog's 'blood vessels,' rings of cells on the outer border of the body-stalk, are found to be tangential sections of the festoons between funnels.

If we recognize this net of angiocyts and solid cords as vascular anlagen, then the presence of such a net in the chorion and body-stalk, unconnected probably in one 1 mm. embryo with the vessels of the yolk-sac, and the presence of a similar though much less extensive net in a younger embryo, where yolk-sac vessels do not exist, shows that blood-vessels arise, which are not only independent of those on the yolk-sac, but even antedate the latter. My observations suggest that they originate from ingrowths of the mesothelium as a number of separated cords, angiocyts, or blood islands, which are soon connected by sprouts of endothelium; that these sprouts may extend along the chorion, as a net, enlarging here and there into angiocyts, and later connecting with the yolk-sac vessels.

Discussed by Schulte.

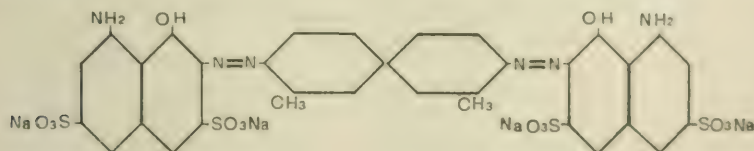
13a. *The relation between chemical constitution, physical properties, and ability of the benzidine dyes to behave as vital stains.* HERBERT M. EVANS, Johns Hopkins University, Research Associate, Carnegie Institute of Washington.

These experiments which will be published in extenso with W. Schulemann have shown in brief that the chemical constitution is of no direct influence on the capacity of the dyes to act as vital stains, but is of indirect importance inasmuch as it affects the physical state of the solution of the dye in water. The entire series of dyes of this class, that is, those made by combining two molecules of an amino-naphthol, naphthol or naphthylamine sulfonic acid with one molecule of a para-diamine base (benzidine, tolodine, dianisidine), act as vital stains in the sense here employed, for they are taken up and stored in the cytoplasm of those cells which react to trypanblue. Many of these dyes, however, do not diffuse sufficiently to give a general vital stain of the cells concerned in the whole body. These more 'negative' dyes are very sensitive to

electrolytes which precipitate aggregated dye particles from them. The ultramicroscopic picture of such negative dyes in contrast to that of trypanblue reveals their greater richness in molecular aggregates and coarser suspended particles incapable of diffusion. The study has revealed the possibility of higher sulfonated combinations with diffusion rates exceeding that of trypanblue. Such dyes, however, are taken into the cell by virtue of the same forces concerned in the reception of the large particles of negative dyes on the part of cells contiguous to them. This is in turn identical with the forces concerned in the reception of larger particles (bacteria, carbon, and so forth) into the cell, an act long known as phagocytosis and having as its basis from the studies of Hamburger and others surface tension alterations. The research consequently extends downwards very appreciably the size of particles which are known to affect the cell and be received into it by virtue of surface or 'adhesive phenomena.' The cells whose protoplasm is especially sensitive in this way form a sharply defined group by themselves and deserve to be classed together with regard to this common peculiarity, that is, that of reacting to particulate matter. The experimental analysis of cells on the basis of their behavior towards various agents is certainly of the greatest worth in tracing the genetic relations and degree of specificity of cell types.

13b. *The physiology of endothelium.*<sup>1</sup> HERBERT M. EVANS, Johns Hopkins University, Research Associate, Carnegie Institution of Washington.

These studies had for their original aim, an analysis of the vital stain obtained by an injection of various benzidine dyes into the blood stream of living animals. The dyes were first used in this connection by Ehrlich and Shiga ('05) and by Nicolle and Mesnil ('06). Trypanblue may be taken as a type of the series. It is formed by the combination of two molecules of 1.8 amidonaphtol 3.6 disulphonic acid with one molecule of ortho-tolodine in alkaline solution, and hence may be represented by the formula:



<sup>1</sup> The work reported is based on more extensive publications (part of which have as yet not appeared) written by the author in collaboration with: (1) Werner Schulemann and Felix Wilborn, "Die vitale Färbung mit sauren Farbstoffen," Jahresbericht D. Schlesischen Gesellschaft für vaterländische Cultur. Naturwissenschaft. Sektion, Sitzung vom 29 Jan., 1913, Breslau, 1913. (2) Werner Schulemann, "The action of vital stains belonging to the benzidine group," Science, 1914. (3) Ibid "The action of acid azo dyes and related bodies."



When 1 per cent aqueous solutions of some of these dyes are injected into the living animals (by the intravenous, intraperitoneal, or subcutaneous route) there results a profuse coloration of the skin, mucous membranes, sclerae, etc., which is not inimical to health and persists for many days. The color imparted soon after injection is due merely to the free diffusion of the dye into the chief body fluids and tissue juices; but after a short time, the dye is taken up by certain cells in sufficient quantity to be seen as distinct 'dye granules' in the cytoplasm. The concentration of the dye in this manner is probably a storage of the dye particles in intracellular localities or depots where it is relatively separate from the living protoplasm and the phenomenon is only shown by living cells, dead cells staining profusely and uniformly. These reasons justify the term 'vital stain.' All of the cells of the body do not behave in this way. In organs, which are mainly epithelial, the vital benzidine dye may be seen only in cells of the connective tissue framework. In common with most epithelia and with the central nervous system, the blood cells remain free from any trace of the stain. On the other hand certain cells react intensively to the dye and become filled with large brilliant granules and vacuoles which mark them out in sharp contrast to their unstained neighbors which have had equal access to the dye. Among these 'vitaly stained' cells two types are predominate: (1) The clasmatocytes (resting wandering cells) of the connective tissues and makrophages of the great serous cavities. (2) The endothelium in certain special localities (liver, lymph glands, bone marrow, spleen).

In addition to the intense reaction of these cells, certain other cells react less intensively to the stain and are normally found with much smaller, often very minute 'granules' of the stain. To such cells belong (1) The fixed connective tissue cells. (2) The mesothelium, for example, lining the peritoneum and covering its organs. (The kidney constitutes an exception to the usual negative behavior of epithelium towards the stain for it shows intense dye granules in the epithelium of its convoluted tubules, especially those of the first order, and in addition free dye in the loops and in various portions of the collecting system so that the stain intensifies the lines of Peter in its gross morphology; the liver cells also accept and store the dye).

The positive behavior of the endothelium in various localities towards the stains, and the negative reaction of the blood cells, offers an unusual opportunity to distinguish these two cells types in various proliferations due to infection, wound-healing, etc. Many experiments of this sort

A monograph. (4) S. J. Crowe, "Studies on the behavior of endothelium." (5) M. C. Winternitz, and F. B. Bowman, "Ueber die vitale Färbung des Tuberkels," *Centralbl. f. Bakteriologie*, I Abt. 65 Bd. 1912, Heft 4, 5. (6) Ibid "An experimental study of the histogenesis of the miliary tubercle in vitaly stained rabbits," *Journal of Experimental Medicine*, 1914. (7) J. T. MacCurdy, "Experimentelle Läsionen des Centralnervensystems, untersucht mit Hilfe der vitalen Färbung," *Ber. Klin. Woch.* 1912, Nr. 36.

have been made and the great proliferative capacity of the endothelium proven. It has been possible to establish beyond a doubt the endothelial nature of the giant cells and epithelioid cells in miliary tubercles. (Evans, Bowman, Winternitz, *Journal of Experimental Medicine*, 1914). Similar clarity has been secured on the active role of endothelium in experimental thrombosis and embolism.

Much interest attaches to the light these studies throw on the normal activities of endothelium. The vital stain shows that the great mononuclear cells which are set free in the lymphatic sinuses of lymph glands, especially in the medullary sinuses, behave identically with the endothelium of these sinuses and in contrast to the behavior of the mononuclear blood cells. They are stained deeply vitally and hence in this respect related to the endothelium. Their actual origin from the endothelium may be seen in all cases where their active formation is called forth. These cells have long been known to pathologists and their endothelial nature championed by various observers, above all by F. B. Mallory. The vital stain establishes this view. These cells are in great abundance in the lymph glands in cases of typhoid fever, anterior poliomyelitis, and a great variety of infections. Their importance in the defense of the body can hardly be exaggerated. It is of interest that, whereas, singularly few of these cells exist in the general circulation, they may under the influence of disease or any excitant to their formation, appear in the general blood stream. They have in fact been seen in the peripheral blood (ear) by various clinical observers without a correct interpretation of their nature being at that time possible (see for example, F. Van Nüys, "An extraordinary blood," *Boston Medical and Surgical Journal*, CLVI, p. 390, 1907; W. B. Bartlett, *Pub. Mass. Gen. Hosp.*, Vol. 2, p. 390, 1908).

The experimental production of such a condition (where these cells, endotheliocytes, exist in the general circulation) can be secured by a long continued injection of the benzidine dyes themselves and in these cases the cells in question are in brilliant contrast to the mononuclear hematogenous elements by virtue of their dye content.

It is highly probable that the endothelial cells of the organs in question, namely, lymph glands, bone-marrow, liver and spleen, are of the greatest importance in the defense against bacterial disease and that they form and set free the so-called anti-bodies in immunity.

Discussed by Bremer and Evans.

14. *Concerning certain cytological characteristics of the erythroblasts in the pig embryo, and the origin of non-nucleated erythrocytes by a process of cytoplasmic constriction.* V. E. EMMEL, Department of Anatomy, Washington University Medical School, St. Louis.

The various views which have arisen in the history of the problem of the origin of the non-nucleated erythrocyte may be briefly stated as including that of intra-cellular nuclear disintegration, nuclear persistence, the hematoblast theory, intra-cellular formation, and the nuclear ex-

trusion theory. With the exception of the hematoblast theory, all of these views are still being more or less seriously discussed, although at the present time that of nuclear extrusion appears to have the greater number of adherents. In contrast to these theories the following results of a study of fresh and fixed blood and blood cultures are apparently indicative of another possible mode of origin for non-nucleated red blood corpuscles.

It was found that the erythroblast of the pig embryo in place of being spherical, as generally described, may in the later stages of cytomorphosis assume a biconcave or cup shape; its nucleus becomes smaller, more compact, eccentric in position, and not infrequently flattened in form; mechanically rotated, the erythroblasts tend to orient themselves with the nuclear region remaining on the under side, as if loaded; and that their reaction to changes in osmotic conditions indicates a structural difference between the nuclear and cytoplasmic poles. These observations were discussed with reference to the question of the correlation of the form of the definitive plastid with the enucleation of the erythroblast, the formation of a lecithin containing membrane, hemoglobin differentiation, and the factors involved in determining the eccentric position of the nucleus.

In some eighty culture experiments non-nucleated erythrocytes or plastids were observed to arise from the parent erythroblast by a process of cytoplasmic constriction. In size, form, hemoglobin content and stain these culture plastids are comparable to the normal circulatory plastids. Observations on living and fixed material indicate the occurrence of a similar process within the embryo. These results accordingly raise the question whether the origin of non-nucleated red blood corpuscles by a process of cytoplasmic constriction rather than by nuclear extrusion or intra-cellular nuclear disintegration does not merit more serious consideration.

A more detailed description and discussion of the data is in press for publication in *The American Journal of Anatomy*.

15. *The formation of red blood cells in the developing thymus of the pig.* J. A. BADERTSCHER, Cornell University.

16. *The relations of mitochondria in cells multiplying by mitotic and amitotic division.* E. V. COWDRY, Johns Hopkins Medical School.

The object was to determine whether there are any changes in the number, shape or cytoplasmic arrangement of mitochondria during cell division.

Material: chick embryos, primitive streak stages to 31 somites. Technique: (1) Meves' iron hematoxylin method; (2) same, with counterstain of erythrosin; (3) Benda's method; (4) Bensley's anilin fuchsin methyl green method; (5) Bensley's anilin fuchsin toluidin blue method; (6) Bensley's anilin fuchsin methylene blue erythrosinate method and (7) janus green intravital.

Results: 1,000 cells were studied by the first method in process of



division; 907 were in mitosis and 93 in apparent amitosis. With regard to mitochondria in mitosis: Out of the 907, 73 showed a relative increase in number, 74 a decrease; of the same cells 4 showed larger mitochondria and 259 more granular ones; 361 out of the 907 cells were in the metaphase; none of them showed mitochondria in the spindle. The observations on amitosis are incomplete.

The general conclusion is that in the material studied, the number, shape and arrangement of mitochondria during mitotic division is essentially the same as in non-dividing cells.

17a. *Ameboid movement in the corial melanophores of frogs.* DAVENPORT HOOKER, Anatomical Laboratory, Medical Department of Yale University.

1. The pigment granules contained within the melanophores of larval and adult frogs are carried in the cell cytoplasm and not in intracellular canals, along rod-like structures nor in a specialized type of protoplasm. They show further, no definite relation or arrangement to one another nor to the nucleus.

2. The melanophores of both larval and adult frogs lie in preformed spaces in the connective tissue and corium, respectively. The melanophores of adult frogs fill the branches of their preformed spaces in the fully expanded phase, those of tadpoles do not.

3. The melanophores of adult frogs have expansion-phase patterns which are constant for each cell and which are forced upon the cells by their preformed spaces.

4. The melanophores of both larval and adult frogs expand and contract within the spaces which enclose them. As the processes of expansion and contraction are performed by means of pseudopodia, these cells are ameboid.

17b. *The development of stellate pigment cells in plasma cultures of frog epidermis.* DAVENPORT HOOKER, From the Anatomical Laboratory, Medical department of Yale University

The prevailing theory in regard to the presence of pigment in the epidermis is that the cells containing it have wandered in from the underlying connective tissue and that the epidermis per se may not elaborate melanine. In this connection the following observations may be of interest.

In Harrison plasma cultures of the epidermis of 3 to 4 mm. embryos of *Rana pipiens*, the elaboration of pigment was observed in some of the epidermal cells. At first appearing as a mass of brown granules in the immediate vicinity of the nucleus, the pigment gradually spread throughout the entire cell. The ratio of pigment-forming cells to those which formed none was about 3 to 1. After the elaboration of a considerable amount of pigment, these cells, either actively or passively migrated to a position below the non pigment-bearing cells. In this position, several assumed a stellate form by sending out pseudopodia. The cultures were at this time four months old, the plasma having been fre-

quently renewed. The cells remained in this condition until the accidental destruction of the preparations, four and a half months from their beginning.

Whether these cells remain as the permanent pigment cells of the adult frog epidermis is uncertain and even questionable, but the observations demonstrate that certain epidermal cells may elaborate pigment within themselves. Harrison observed the formation of pigment in cells of the medullary tube in lymph cultures. Study of tissues, especially *in vitro*, demonstrates that the ability to form pigment is normally very widespread throughout embryonic development.

18. *Vital staining of the interstitial cells of the testis.* R. H. WHITEHEAD, Anatomical Laboratory of the University of Virginia.

The theory advanced thirty years ago by v. Bardeleben that the interstitial cells of the testis are capable of passing through the walls of the seminiferous tubules and there function as Sertoli cells received no support from subsequent students of the subject until quite recently. The late E. Goldmann (*Die äussere u. innere Sekretion in Lichte der 'vitalen Färbung,'* Tuebingen, '09) in the course of an extensive study of various tissues and organs by vital staining, especially with pyrrholblau, investigated the interstitial cells of the testis. He injected subcutaneously into white mice 10 cc. of a 1 per cent watery solution of this dye every two or three days for ten days, and after killing the animals examined the tissues for the most part in frozen sections. The interstitial cells take the dye and so are readily recognized; the epithelium of the tubules is quite free from it. He states that the interstitial cells can be observed in all stages of migration up to complete entrance within the tubules. His observations were subsequently confirmed by J. Kyrle (*Ueber die Regenerationsvorgaenge in tierischen u. menschlichen Hoden*, Wien, '11).

It is difficult to see how, if the observations of these investigators are correct, this behavior of the interstitial cells could have escaped the many who have studied them in innumerable sections; and it seemed worth while to repeat the experiments, relying, however, upon thin sections of imbedded material rather than upon frozen sections. The organs were fixed in 10 per cent formalin, dehydrated in acetone (the dye is quite soluble in alcohol and in water), cleared in xylol, imbedded in paraffin, and sectioned at 7 micra. Congo red was used as a counter stain; it stains quickly and brings out the walls of the tubules quite distinctly.

In such sections the interstitial cells are not all stained with the pyrrholblau, but cells containing the blue grains are sufficiently numerous to allow conclusions. In none of the sections have I been able to see any evidence of the migration of interstitial cells into the tubules. Accordingly I conclude that the observations of Goldmann need further confirmation before they can be accepted.

TUESDAY, DECEMBER 30TH, 9.30 A. M. to 12.00 M. SESSION FOR THE READING OF PAPERS, PRESIDENT ROSS G. HARRISON AND VICE PRESIDENT THOMAS G. LEE, PRESIDING.

19. *The development and growth of the incisor teeth of the albino rat.* WILLIAM H. F. ADDISON AND J. L. APPLETON, University of Pennsylvania, Philadelphia, Pa.

20. *Development of the pancreatic duct-system in the pig.* GEORGE W. CORNER, Johns Hopkins University.

The author has injected the pancreatic ducts of pig embryos, and reports the following observations: The youngest stage at which the injection of the ducts is possible is at 30 mm. total length. At this time the ducts form a capillary plexus with frequent anastomoses. At 40 mm. certain strands of the plexus begin to dilate, and at 50 mm. they have formed a well-marked main-duct. The picture so closely resembles the formation of blood-vessels from plexuses that it is suggested that there may be a flow through the pancreas at an early stage, thus bringing about the formation of the main duct-channel (In discussing the paper, Professor Bensley confirmed the statement that the pancreas secretes at a very early period). At 60 mm. there begin to grow out from the anastomosing capillaries little branching, non-anastomosing twigs, similar to those found by Laguesse in the sheep (by reconstruction from sections) and called by him primitive vesicles. These twigs replace the plexus, and grow into the great duct-tree of the adult. Above 110 mm. no anastomoses between ducts may be found by the injection method. This work will be published in full as part of a paper on "The structural unit and growth of the pig's pancreas."

Discussed by Bensley, Scammon and Corner.

21. *On the pelvis of the human embryo.* JOHN WARREN.

Two features of the development of the human pelvis were presented at the meeting of Anatomists in December—one, the early development of the inguinal region and the formation of the gubernaculum testis, and secondly, the early development of the muscles of the perineum and pelvic floor. Observations were made on human embryos of 18 mm., 19.3 mm., 22.8 mm., 29 mm., 37 mm., and 42 mm. head-rump length, all taken from the Harvard Embryological Collection. The first appearance of the gubernaculum testis was observed in an embryo of 18 mm. It appears at first as a slight thickening in the lateral wall of the abdomen before there is any trace of the abdominal musculature. This thickening forms a crest in the ventro-lateral wall of the abdomen, the inguinal crest, which becomes attached to the lateral portion of the urogenital fold, and in this way a small recess in the coelom is cut off behind the inguinal crest. In an embryo of 19 mm. the mass of connective tissue which represents the gubernaculum is more clearly differentiated.



and it streams out into the connective tissue of the abdominal wall without any very distinct peripheral limits. In an embryo of 22.8 mm. the three layers of the abdominal musculature are now distinct. The gubernaculum can be clearly differentiated from them and its peripheral end is already presenting at the future external abdominal ring. The ridge formed by the gubernaculum on the ventro-lateral side of the abdominal wall is very marked and in frontal section the gubernaculum appears as a roundish mass of mesenchyme with fairly distinct outlines. In an embryo of 29 mm. essentially the same conditions are found, but we have here the first traces of a processus vaginalis, which extends through two or three sections on the mesial aspect of the gubernaculum, forming a tiny pouch penetrating the substance of the abdominal wall. The elevation formed by the gubernaculum over the ventral-lateral aspect of the abdominal wall is very striking. In an embryo of 42 mm. the gubernaculum appears as a large oval mass of tissue entering the abdominal wall at the internal abdominal ring, which is clearly differentiated. The processus vaginalis extends through a dozen or fifteen sections, being found principally on the mesial aspect of the gubernaculum, and also for a limited extent on its lateral aspect. At the external abdominal ring the gubernaculum becomes directly continuous with a fan-shaped mass of mesenchyme which spreads up and down in the subcutaneous tissue of the lower part of the abdominal wall. This represents the ligamentum scroti, and extends down to the base of the future scrotal folds. As regards the development of the muscles of the perineum and of the pelvic floor, the first distinct traces of the levator ani muscle and of the external sphincter muscle of the rectum could be clearly observed in the 18. mm. embryo. They appeared as thickenings in the mesenchyme and were only fairly well differentiated from the surrounding tissue. In the 22.8 mm. embryo the two muscles were very well marked. The levator ani muscle especially shows at this stage almost its exact adult relations to the rectum and to the genitalia. The topographical position of the future ischio-rectal fossa was very clearly defined. No trace could be observed at this stage of the muscles of the perineum, though the perineal nerves and vesicles were very distinct. In the 29. mm. embryo the levator ani and the external sphincter were essentially the same as in the previous stage. The first trace of the ischiocavernosus muscle and of the bulbocavernosus muscle appeared as a thin superficial layer of fibers, lying in the first instance on the mesial aspect of the primitive crus penis, and on the lateral aspect of the future spongy portion of the penis. In the 37. mm. embryo all these muscles were very sharply defined. The external sphincter of the rectum formed a suprisingly thick ring-like mass of muscle, surrounding the lower end of the rectum. The levator ani was distinctly divided into two parts,—an anterior part, fairly thin which covered over the lateral wall of the rectum, and a thicker, rounded, posterior portion, which had no direct relation to the rectum. The bulbocavernosus and ischiocavernosus muscles were very clearly outlined and the perineal nerves could be traced directly into them. Only a very ill-defined con-

densation of mesenchyme gave a slight hint of the triangular ligament and of the transversus perinei muscle, these layers being apparently developed later than this stage.

22. *A case of hemocerebellar atrophy in a child.* OLIVER S. STRONG, Anatomical Laboratory, Columbia University.

The clinical symptoms were not carefully studied but the following were communicated, from memory, by the physician in charge.

The child was three years and four months old. It was small, its head was small and all its movements weak and unsteady. It sat up all day in a high-backed chair. It could walk, but with a very uncertain gait, staggering, and with a tendency to hold fast to chairs. It was unsteady in grasping a proffered object. It could move its head slowly, and continuously moved it from side to side, usually humming a tune (without words). It had a marked bilateral nystagmus, exact type not noted. It was mentally very weak, appeared very dull, took little interest in toys, and so forth. It talked poorly and indistinctly, with scarcely any formed sentences. Death was due to measles and broncho-pneumonia.

Inspection of the external surface of the brain showed the following: The left hemisphere of the cerebellum was entirely absent except a small lobe apparently representing the flocculus. The median lobe was present, though in a defect of this kind, obviously congenital, an exact identification of parts is difficult. The right olive was apparently entirely absent, the left olive normal. The cranial nerves were apparently normal. The pons was very asymmetrical, the transverse fibers and middle peduncle were normal on the right side but enormously reduced on the left side, so much reduced that the VII and V nerves issued in immediate contiguity with each other. The pons protruded much more on the left side. The left pes was wider than the right.

A dorsal view of the brain stem, with the cerebellum removed, showed a curvature of the median line with the convexity toward the left. The left clava and cuneus were longer than the right, extending further cephalad. The two trigona hypoglossi were nearly symmetrical, the left ala cinerea, the left eminentia abducentis and the left trigonum acustici also extended further cephalad than the right. This asymmetry of clavae, cunei, alae cinerae and trigona acustici would apparently be due to the unequal pressure upon the medulla of the asymmetrical cerebellum. The left corpus restiforme was much smaller than the right.

The right superior peduncle was much larger than the left. Some transverse cuts made through the cerebellum did not reveal any left nucleus dentatus. The inferior colliculi were asymmetrical, the left being narrower, more prominent and protruding farther caudad and its brachium appearing less prominent than that of the right. The left superior colliculus appeared to be largely lacking. There appeared to be some atrophy in the posterior frontal, central and possibly part of the parietal lobes of the right cerebral hemisphere.

Sections were made and stained by the Weigert-Pal method, somewhat modified. They showed the following:



In the cervical cord no asymmetry was noted. The spinocerebellar tracts are present on both sides and the lighter areas, usually taken as marking the location of Helwig's tracts, are present on both sides. There is no evidence of an absence of a rubro-spinal tract on one side but this asymmetry probably would not be discernible if present.

In the medulla, whether there is an asymmetry of the arcuate nuclei is somewhat doubtful. No asymmetry was observed in the external nuclei of the columns of Burdach nor was there any noticeable asymmetry in the lateral nuclei. All of these three structures then, as far as they are connected with the cerebellum, would either be connected equally with each half or only with the median lobe and flocculus.

The left olive is perhaps somewhat hypertrophied. The right olive is represented by a small U-shaped mass of gray without minor folds which extends from about the same level caudally to nearly the same level cephalad as the left olive. The caudal part of this atrophic right olive is the larger and there can also be made out vestiges of a median and dorsal accessory olive. The olivo-cerebellar fibers from left olive to right cerebellum are conspicuous, those from right olive to left cerebellum nearly absent. It is obvious that the olive is almost entirely or, not improbably, entirely connected with the opposite half of the cerebellum, for the left flocculus-like lobe and left half of the median lobe would be sufficient to account for the presence of the small right olive. The left central tegmental tract is much more conspicuous than the right. The right medial lemniscus is larger than the left. The reason for this is not entirely apparent. The right nuclei pontis are largely but not entirely absent. There is a small, atrophic left middle cerebellar peduncle. On the other hand the left nuclei pontis appear to be, perhaps, somewhat hypertrophied. The vicinity of the medial lemniscus is invaded by masses of gray apparently connected with pontile fibers and representing aberrant pontile nuclei. Either from these nuclei or from the left pons, bundles of fibers resembling transverse pontile fibers cross the median line in company with the trapezium fibers and appear to join the opposite middle cerebellar peduncle. These fibers would appear to be aberrant transverse pontile fibers. There could not be detected any marked asymmetry of the perpendicular pontile fibers. The left crura are about three times as wide as the right, indicating the absence of the pallio-pontile fibers in the latter. The substantia nigra is correspondingly unequal. The central gray of the right locus coeruleus is thicker than the left and contains a number of bundles of aberrant fibers. These fibers appear to pass caudad to a region where it would seem they must be connected with the juxta-restiform body but this could not be determined with certainty owing to defects in the series. These fibers also pass into the reticular formation accompanying or connected with peculiar streaks of gray passing through the reticular formation ventro-laterally. Some of these fibers possibly join the pons. Further cephalad, in the isthmus, a conspicuous bundle emerges from the central gray of the right floor of the ventricle and passes ventrally in the raphé, possibly entering the pons.



There is a minute left nucleus dentatus and a very small left superior peduncle. The practical absence of this peduncle causes a marked asymmetry in the arrangement of the lemnisci of the two sides. The right nucleus ruber is largely absent, whether completely absent could not be established owing to a defect in the series.

From the above it is evident that the afferent pallioponto-cerebellar path to the left cerebellar hemisphere and the efferent dentato-rubral path from the left cerebellar hemisphere are nearly entirely absent. Of the inferior peduncle, those spino-cerebellar connections, whether interrupted in cord or bulb, which are supposed to be afferent to the median lobe appear to be practically intact; the olivo-cerebellar part of the inferior peduncle however, is nearly absent and must be regarded as passing principally or entirely to the hemisphere, in accordance with the work of other recent investigators

Discussed by B. D. Myers and Harrison.

23. *The morphology and development of the floor of the interbrain in mammals.* FREDERICK TILNEY, From the Department of Anatomy, Columbia University.

The object of this paper is to present a study of the floor of the inter-brain in mammals by means of the reconstruction method. This method was applied to several forms, among which were the adult cat, dog, rat, and rabbit. Observations were also made upon serial sections obtained from a number of other mammals including marsupials, rodents, ungulates, carnivores, primates and man. In the light of this study the third ventricle reveals itself as a more complex chamber of the brain than would appear from the usual description of it. Its complexity is due to the presence of several accessory recesses, each of which is indicated upon the surface by an eminence or protuberance. Some of these structures have previously been recognized but their phylogenetic significance has not been altogether clear. By reconstructions demonstrating the development of the diencephalon in the cat, it was possible to trace the ontogenetic history of each element in the floor of the ventricle and in this way homologize the structures in the basal part of the mammalian interbrain with those in the same region of the selachian brain.

A reconstruction model of the ventricular floor of the adult domestic cat shows the following recesses and eminences, enumerated from the optic chiasm caudad toward the mamillary body.

<i>Accessory recess</i>	<i>Surface eminence</i>
Recessus praeopticus } .....	crista supraoptica
Recessus intraopticus }	
Recessus tuberculi.....	eminentia saccularis
Recessus infundibuli.....	infundibulum
Recessus processus infundibuli.....	processus infundibuli
Recessus premammillaris.....	eminentia premammillaris

Several eminences appear on or adjacent to the floor, but contain no recesses. They are the eminentiae laterales (protuberant lateral portions of the tuber cinereum) the corpora mammillaria and the interpeduncular ganglion.

The development of all of the above structures depends upon changes in three distinct areas of the interbrain. As they appear in the 19 somite cat embryo these areas are the hypencephalon of Kupffer, the primitive optic groove and the lamina terminalis. The more important changes occur in the hypencephalon. This region in the 4 mm. embryo (about 26 somites) is divided by a ridge in such a way as to form a dorsal and a ventral sac.

Von Kuffner has shown similar sacs in the development of selachians, ganoids, teleosts and amphibians. Corresponding evaginations have also been observed in the hypencephalon of sauropsids. In fish the dorsal sac gives rise to the posterior lobes, while from the ventral sac arise the inferior lobes and the saccus vasculosus.

In the 10 mm. cat embryo two small evaginations have made their appearance dorsal to the dorsal sac. The more ventral of these two evaginations becomes the corpora mammillaria, the more dorsal, the ganglion interpedunculare.

Later stages in the development of the cat up to 70 mm. shows that the ventral sac gives rise to saccular eminence, infundibulum and infundibular process. From the dorsal sac develops the praemammillary eminence. This latter eminence is a conspicuous feature of the floor of the third ventricle in such forms as the lion, grizzly bear and leopard. It is present in all the mammals examined by the writer and, although somewhat obscured in the adult human brain, is well marked in the child.

The evidence presented seems to justify the following homologies:

<i>Mammalian brain</i>	<i>Selachian brain</i>
Eminentia saccularis.....	lobi inferiores
Infundibulum and processus infundibulum .....	saccus vasculosus
Eminentiae laterales.....	lobi laterales
Eminentia praemammillaris.....	lobi posteriores

The intraoptic recess which communicates with the third ventricle and extends for a considerable distance through the optic chiasm into the optic nerve is the remnant of the primitive optic recess. Its clinical significance in its possible connection with choked disc due to increased intra-cranial pressure is, at least, suggestive. This problem will require further observations upon pathological material as well as experimental controls, the result of which will be reported in a subsequent paper.

24. *On the so-called 'Bulbar' portion of the Accessory nerve.* D. DAVIDSON BLACK, Anatomical Department, Medical School, Western Reserve University

The following observations have been made from a series of transverse sections through the medulla and upper three cervical segments of a new-born babe, prepared by the pyridine Cajal method of Ransom.

This series represents a part of the material prepared in the course of a study of the calamus region which is as yet incomplete. However, certain facts bearing upon the relations of the so-called 'bulbar portion' of the nervus accessorius have been noted. These are of interest when contrasted with the usual description of the origin and relations of this structure obtaining in current texts.

The origin of the spinal portion of N. XI has been definitely established and can be made out quite well in this series. The nucleus occupies a central and somewhat lateral position in the anterior horn in the cord, and extends upwards into the medulla to about the level of the lower third of the pyramidal decussation. The cells are of typical somatic type, and the emergent fibers pass to the periphery in the well known geniculate manner, so that in no transverse section is the whole course of these fibers displayed.

The ventro-mesial cell group of the anterior horn may be traced as a practically uninterrupted column into the hypoglossal nucleus.

Laterally the cells of the anterior horn become scattered, and lose their characteristic grouping when traced into the formatio reticularis of the medulla.

There is a very apparent interval between the cephalic extremity of the cervical accessory nucleus and the caudal end of the ambiguous cell group.

The dorsal nucleus of the vagus may be traced as a very distinct cell column almost to the lower end of the pyramidal decussation. In other words, this nucleus overlaps that of the accessory nerve in the lower medulla.

There is thus a space between the cephalic extremity of the nucleus of the accessory nerve in the cord and the lower end of the cell group usually described as giving rise to its bulbar fibers, namely, the nucleus ambiguus.

In the interval, in the series described, there are numerous fibers to be seen taking their origin direct in the dorsal nucleus of the vagus, and passing to the periphery ventral to the substantia gelatinosa Rolandi. In their emergent course these fibers are curved laterally and caudally so that in a transverse section their whole extent is not seen. These fibers presumably make up the caudal portion of what is usually described as the bulbar part of N. XI.

At a higher level, where the nucleus ambiguus becomes definitely recognizable, fibers arising from this source take the well known indirect course to the periphery, joining on their way fibers from the dorsal vagal nucleus, and passing *ventral* to the substantia gelatinosa Rolandi. This last point is the only one in which these fibers differ from those usually described as giving rise to the vagus proper.

Conclusions: (1) There is no morphological ground for the consideration of the bulbar XI apart from the vagus in the specimen I have studied; its nucleus of origin is but the caudal prolongation of the dorsal vagal nucleus. (2) That Kolliker's distinction between the emergent fibers of the bulbar XI and those of IX and X, based on the observations



that those of the former pass out ventral, while the latter pass through or dorsal to the substantia gelatinosa Rolandi, is without significance. (3) The extent of the vagal and accessory nuclei corresponds practically to Kappers' findings in *Didelphys*.

In view of the recent investigations of Van Gehuchten, Molhant, Ranson, Kappers, Malone and others, together with the above observations, would it not be better to consider this structure as part of the vagus proper and restrict the term *nervus accessorius* to the present spinal portion of this nerve?

Discussed by Streeter.

25. *Further observations on the sound-transmitting apparatus in Urodeles.* H. D. REED, Zoölogical Laboratory, Cornell University.

In 1909 Kingsbury and Reed<sup>1</sup> published a paper in which they stated that typically the sound transmitting apparatus in *Urodeles* is composed of two elements appearing at different ages of the individual and differing in origin. The element which they called 'columella' is the first to arise during development and is wholly extraotic in origin. It appears as a cord of cells which is connected from the very outset with the squamosum. The ventral end of the cord reaches the middle of the fenestra vestibuli where it spreads out into a broad plate which becomes jointed to the fenestral membrane. The other element called 'operculum' does not arise until just before transformation. It is otic in origin since the major part, at least, is cut out from the walls of the ear capsule caudad of the foramen. It acquires connection with the suprascapula through the M. opercularis. After the operculum arises the columella fuses to a greater or lesser extent with the cephalic lips of the fenestra but retains its connection with the suspensorium through the stylus and its ligament.

Certain forms, as for example *Triton*, *Diemictylus*, the *Plethodontidae*, *Desmognathidae* and *Amphiumidae*, were found to possess but a single element in the fenestra vestibuli. In *Triton* and *Diemictylus* this element possesses the connection with the suprascapula through the M. opercularis and in every respect of structure and relations it is identical with the operculum of the typical forms and development shows this to be true. The columella very early in larval life fuses completely with the cephalic lips of the fenestra.

In the *Plethodontidae*, *Desmognathidae* and *Amphiumidae* the single fenestral element has the structure and relations of both columella and operculum. The cephalic portion of the plate possesses a stylus which is connected with the suspensorium while in the caudal portion is found the perilymphatic prominence with a muscle extending to the suprascapula. The cephalic part of the plate is always fused at its ventral angle with the lips of the fenestra.

<sup>1</sup> B. F. Kingsbury and H. D. Reed. The columella auris in *Amphibia*. Jour. Morph., vol. 20, 1909, pp. 549-628.

The natural inference is that the single element in these forms represents a fusion of the two elements, columella and operculum. A study of complete developmental stages shows that this is true but the greater part of the fenestral plate represents operculum or at least tissue which is otic in origin.

A brief description of the development in *Spelerpes bislineatus* will illustrate the conditions in all. The fenestra in *Spelerpes* is morphologically larger than in *Ambystoma* since it represents both the primary and secondary fenestrae of that form. The columella arises in the typical fashion but instead of spreading out to form a plate which fits into the fenestra it remains as a cylinder of cells extending horizontally across the fenestral membrane and when fully chondrified does not increase in size or in any way spread out upon the membrane. The connection with the ear capsule is very early established by the upward growth of the lips of the fenestra. In the caudal portion of the fenestral membrane which corresponds in relative position to the operculum of *Ambystoma* separate centers of chondrification arise and through growth eventually meet. In this way there is formed a sieve-like plate which fuses with the extraotic rod of cells. Later it becomes completely chondrified. The fully formed plate, therefore, represents two elements, which, while fused in the definitive structure, are distinct in their origin. The extraotic rod of cells becomes the stylus of the fenestral plate and constitutes the representative of the columella in these forms. The fenestral plate, while in no part is cut out from the ear capsule, is otic in origin and to be regarded as the equivalent of the operculum.

The conditions as described above in *Spelerpes* are those which prevail in the *Desmognathidae* and *Amphiumidae* in all of which a single fenestral element is present. *Siren* possesses but a single fenestral element which is lacking in both suspensorial and shoulder girdle connections. The need of developmental stages for study leaves the nature of the plate unsettled.

26. *The tendency toward adjustment of posture in transplanted labyrinths*,  
G. L. STREETER. University of Michigan (This paper will be printed in full in *The Journal of Experimental Zoölogy*, vol. 16, 1914).

27. *On the development, attachment and action of the tectorial membrane*.  
IRVING HARDESTY, Tulane University.

The beginning of the tectorial membrane appears in foetal pigs of 3 to 5 cm. long as an imperfectly fibrous, transparent film lying upon and produced by a thickening of the epithelium of the foetal cochlear duct along its axio-basal aspect. This band of thicker epithelium becomes the "greater epithelial ridge" of the later stages.

In foetuses of 6 to 9 cm., the greater ridge has become thicker and broader and appears only in the base of the cochlear duct, due to the invasion of its axial side by the mesenchymal syncytium to form the vestibular lip of the spiral lamina. As this invasion proceeds, the axial cells cease to produce tectorial membrane and thus the axial edge of the

membrane remains thin and is held adherent upon the vestibular lip. The now thicker film upon the greater ridge shows structure characteristic of the tectorial membrane. At the extreme lateral or outer margin of the greater ridge there is differentiating a line of cells, 2 or 3 wide, which cells are broader than their neighbors and when traced through the later stages become the rods of the organ of Corti, these cells and a few others about them increasing in height to form the "lesser epithelial ridge" (anklage of the organ of Corti) lateral to the greater ridge and tectorial membrane.

The greater ridge increases in both width and thickness, acquiring its maximum width in foetuses from 13 to 16 cm. Its cells are steadily contributing thickness to the basal side of the tectorial membrane, connected with it by 3 to 6 delicate fibers continuous from the distal end of each cell. The lateral or outer edge of the developing tectorial membrane here conforms closely to the rounded lateral margin of the greater ridge, cupping around this margin and fitting into the groove between it and the lesser ridge, the basal cells of this rounded margin coming to lie at almost right angles to the axis of the cochlea in order to be perpendicular to the edge of the membrane they are forming.

During the earlier stages of their differentiation, the cells of the lesser ridge (beginning organ of Corti) likewise produce a few filamentous threads and these threads are seen extending from their cells of origin and adhering to the vestibular surface of the outer edge of the developing tectorial membrane. These threads disintegrate in the later stages and thus contribute no part of the adult tectorial membrane. In structure, the tectorial membrane consists of a hyaline matrix, probably keratin, in gelatinous form, in which are imbedded the very numerous fine fibres or threads of uniform size, the varying directions of which are determined by the varying directions of the cells producing the membrane during the different stages of the increase and decrease of the greater ridge. The filaments produced by the cells of the early lesser ridge have ceased to grow in pigs of 16 cm., are never embedded in a matrix as are those of the tectorial membrane, are largely disintegrated at 19 cm., and have totally disappeared in foetuses near term.

In pigs from 13 to 16 cm. the cells forming the axial side of the greater ridge begin to decrease in height and number. Beginning at this axial margin, the cells gradually become divorced from the membrane they have produced, the process of divorce proceeding outwardly, the divorced cells receding and decreasing in number till they become the fewer, flattened cells lining the spiral sulcus of the adult. The last cells to become separated from their product are thus those immediately adjacent to the inner sustentacular cells of the organ of Corti. The recession and decrease in number is accompanied by an appreciable narrowing of the basal floor of the spiral sulcus. In foetuses of 15 cm., the width of the greater ridge may measure in the apical coils of the cochlea twice the width of the basal floor of the spiral sulcus in the adult. In the basal coil this decrease in width is only about one third of the width at 15 cm.



This developmental decrease of the distance between the organ of Corti and the vestibular lip of the spiral lamina results in the change in position of the organ of Corti with reference to the tympanic surface of the tectorial membrane and, of course, in a tearing free of the membrane from any attachment it may have with structures lateral to the vestibular lip of the spiral lamina. In the apical coils the membrane of the adult may come to extend not only over the entire organ of Corti but also over from 9 to 13 of the cells of Claudius. Thus the tectorial membrane is only attached along its axial edge upon the vestibular lip of the spiral lamina. Its outspanning portion is of necessity free.

From various measurements taken from 9 growth stages and from the adult, the most probable explanation of the final position, well under the tectorial membrane, acquired by the organ of Corti is that it is due to a growth in width of the vestibular portion of the spiral lamina resulting in an outer or lateral projection of the membrane over the organ, and in greater part to an actual shifting axialward of the organ of Corti coincident with the disintegration of the greater ridge.

In the adult pig the tectorial membrane is about 30 mm. long. In the apical turn it is about 5 times as wide and 5 times as thick as is its basal end; its area in section in the apical turn is approximately 21 times and its volume 95 times the area in section and the volume of its basal end.

Teased fresh specimens of the tectorial membrane show that it decreases evenly from its larger, apical towards its smaller basal end and it has sufficient elasticity, probably due to the arrangement of its fibers, to maintain its position over and approximate to the organ of Corti. Longitudinally it is exceedingly flexible, offering practically no resistance to stress applied transversely to its long axis.

An apparatus constructed to simulate the essential parts of the auditory apparatus is thought to indicate something of its functional action. The external meatus is represented by a large mouthed megaphone over the smaller end of which is stretched a piece of gold beater's skin for the tympanic membrane. The cochlear duct is a narrow, thick-walled box, 42 inches long, with a wooden spiral lamina and thin wooden basilar membrane and with drum-skin covered openings representing the fenestrae, at the basal ends of the scalae. The tectorial membrane is represented by a piece of very thick elk's hide pared to the shape and approximate proportions of the membrane and softened, and its thin edge affixed upon the vestibular lip of the spiral lamina. At 6 equal intervals fine platinum wires are passed through the membrane to make contact below with small copper plates representing the organ of Corti, the plates being continuous with wires and capable of being raised or lowered by means of adjustment screws beneath the box. Batteries are connected with the platinum and copper wires at each interval. The box, which has a water-tight, glass top, is completely filled with distilled water, the top fastened down without inclusion of air, and a representative of the ossicles is placed pressing between the tympanic membrane and the skin representing the fenestra ovalis. Telephones are interposed in the circuits made by contact of the platinum wires with the copper plates.

Ordinary organ reeds, having known vibration frequencies, were used chiefly in the experiments. These were sounded into the megaphone. It was found that any vibration from a step upon the laboratory floor up to the note G below 'middle C' (196 vibrations per second) would throw the membrane into vibration throughout its entire length. This note caused the membrane to vibrate more strongly at the fourth interval from its smaller end than at any other interval, indicating a certain amount of resonance in this region. A, the next note above, produced vibrations at all intervals of the membrane except the sixth, the apical end. Middle C, 261 vibrations per second, was damped out at the fourth interval. Notes above F, which is 349 vibrations per second, produced vibrations in no part of the membrane. Occasionally a note other than G produced more pronounced vibrations at a certain interval than at others but evidence of the existence of resonance were unsatisfactory.

From results with this comparatively coarse and crudely constructed apparatus, it is suggested that notes up to a certain pitch throw the entire natural tectorial membrane into vibrations of corresponding frequencies and that sensations of pitch are determined by the frequency of impingement of the membrane upon the auditory hairs, intensity being determined by the amplitude and quality by the quality of the wave motion imparted. Further, that the highest notes within the range of the auditory apparatus throw, according to their frequency, only varying extents of the smaller, basal end of the tectorial membrane into vibration, being so damped out in passing toward the apex of the cochlea, overcoming friction, the inertia of the endolymph and that of the membrane itself, as not to produce vibrations in the heavier, apical portions. Finally, since, if the tectorial membrane varying in mass as it does were cut into a number of segments, each segment would have a different natural vibration frequency, it is possible that it exercises a certain amount of resonance. The diaphragm of the telephone possesses a small amount of resonance. The above results suggest a modification of the telephone theory of hearing.

WEDNESDAY, DECEMBER 31, 9.30 A.M. to 1.00 P.M. SESSION FOR THE READING OF PAPERS. PRESIDENT ROSS G. HARRISON AND VICE PRESIDENT THOMAS G. LEE, PRESIDING.

28. *Regeneration of medullated nerves in the absence of the embryonic nerve fiber following experimental non-traumatic degeneration.* ELBERT CLARK, Hull Anatomical Laboratory, University of Chicago.

In this study degeneration of the medullated nerves was brought about in the domestic fowls by a prolonged exclusive feeding of polished rice (finest quality of white table rice). There frequently resulted a pronounced paralysis of the legs which was always accompanied by marked degeneration in the medullated fibers of the sciatic nerve. Recovery of the fowls and regeneration of the nerves was accomplished by returning the fowls to an adequate nutritive diet.

In such fowls the nerve fibers are intact during degeneration and all traumatic and inflammatory effect produced by cutting the tissues and the nerve or of tying the latter are obviated; the process of degeneration can be stopped at almost any stage or greatly prolonged, and several stages of degeneration are to be observed in different fibers of the same nerve. In regeneration the possibility of an ingrowth of fibers from other nerves into the regenerating nerve under observation is eliminated and repair of the medullated nerves can be induced after any stage of degeneration.

Ten to twenty percent of the medullated fibers of the nervus ischiadicus showed a complete fatty change of their medullated sheaths into globules of degenerated myelin and a segmentation or granulation of their axis cylinders. No multiplication of the nuclei of the neurilemma sheath could be observed and consequently no "embryonic nerve fibers" or "Band-fasern."

During recovery these degenerated fibers attained new axis cylinders and the medullary sheaths returned to normal. In other words, regeneration has been observed to follow degeneration in medullated nerve fibers without passing through the embryonic nerve fiber or Band-faser stage.

By prolonging the degenerative process there resulted a multiplication of the nuclei of the neurilemma sheath. This and other experiments tend to show that the embryonic nerve fiber may be coincident with a late stage of degeneration. It may not represent an early stage of regeneration and its presence does not signify an attempt at regeneration on the part of the medullated nerve fiber.

In the absence of the embryonic nerve fiber, the degenerated myelin was absorbed with extreme slowness, persisting as droplets after one year and fourteen days. On the other hand, where the embryonic nerve fiber was formed the degenerated myelin quickly disappeared from the fiber. The conclusion is reached that the proliferating nuclei of the neurilemma sheath participate in the resorption of the degenerated myelin.

In regeneration a new axis cylinder was attained by outgrowth and in the absence of the embryonic nerve fiber. The new axis cylinder grew down the old medullary sheath which latter still contained large globules of degenerated myelin and fragments of the old axis cylinder. The outgrowing axis cylinder was seen to branch, and in cross-sections of the nerves two new axis cylinders were observed within the same old medullary sheath. The embryonic nerve fiber could, of course, play no part in the formation of the new axis cylinder, either by autoregeneration or by outgrowth.

No indications of regeneration were observed in the fibers of the spinal cord.

Discussed by Sheldon.

29. *Some changes in the nervous system of the metamorphosing tadpoles of Rana pipiens.* ELIZABETH H. DUNN, Woods Hole, Mass.



30. *The development of the cranial sympathetic ganglia. A comparative study.* ALBERT KUNTZ, St. Louis University School of Medicine.

The observations on which the following conclusions regarding the development of the cranial sympathetic ganglia in fishes are based were made on preparations of embryos of the common toad fish (*Opsanus tau*.) The six sympathetic ganglia on the cranial portion of the sympathetic trunk are genetically related to the I spinal nerve and the X, VII, and V cranial nerves. The majority of the cells giving rise to these ganglia are derived directly from the I spinal ganglion and the cerebral ganglia associated with the X, VII, and V cranial nerves. Certain of these sympathetic ganglia receive cells also which advance peripherally from the wall of the neural tube along the fibers of the motor nerve roots. The ciliary ganglion arises in the path of the oculomotor nerve. It is derived primarily from cells which advance peripherally from the wall of the mid-brain along the fibers of this nerve. As development advances the ciliary ganglion becomes connected with the Gasserian ganglion and the first sympathetic ganglion associated with the latter, through the radix ciliaris longa. After this connection is established, a relatively small number of cells which wander out from the Gasserian and the first sympathetic ganglia are, doubtless contributed to the ciliary ganglion.

In larvae of *Amblystoma* and *Rana* the ciliary ganglion bears the same genetic relationship to the oculomotor nerve and arises in essentially the same manner as in embryos of *Opsanus*. The cranial division of the sympathetic nervous system is relatively feebly developed in the Amphibia. The ciliary ganglion is relatively small in the larvae of both *Amblystoma* and *Rana*. Other distinct sympathetic ganglia probably do not occur in the cranial region in these types of Amphibia. However, sympathetic ganglion cells are incorporated in, or associated with, certain of the cerebral ganglia.

In embryos of the turtle, the ciliary ganglion arises at the growing tip of the oculomotor nerve. The majority of the cells which take part in the development of this ganglion have their origin in the wall of the mid-brain and advance peripherally along the oculomotor nerve, or are the direct descendants of such cells. As development advances, the ciliary ganglion becomes connected by a fibrous ramus with the ophthalmic division of the trigeminal nerve. After this connection is established, a relatively small number of cells which advance peripherally from the Gasserian ganglion are contributed to the ciliary ganglion.

The sphenopalatine ganglion arises, in embryos of the turtle, in the path of the great superficial petrosal nerve and soon becomes connected by fibrous rami with the maxillary division of the trigeminal nerve. It is derived from cells which advance peripherally from the geniculate ganglion and the Gasserian ganglion respectively along the great superficial petrosal and the maxillary nerves.

Ganglia homologous with the otic and the submaxillary ganglia of the higher vertebrates were not observed in embryos of the turtle.

In embryos of the chick the ciliary ganglion bears the same genetic relationships to the oculomotor and the ophthalmic nerves and arises in essentially the same manner as in embryos of the turtle.

The otic ganglion arises, in embryos of the chick, in the path of a tract of sympathetic fibers which emerge, at the level of the geniculate ganglion, from the sympathetic plexus surrounding the carotid artery and continue cephalad. It is derived primarily from cells which advance cephalad from the superior cervical ganglion and cells which wander out from the geniculate ganglion.

In proximity with the olfactory epithelium, in embryos of the chick, is located a relatively large ganglion which is primarily related to the great superficial petrosal nerve, but has fibrous connections also with the maxillary nerve. This ganglion, described by Rubaschkin<sup>1</sup> as being related to the trigeminal nerve, is probably homologous with the sphenopalatine ganglion of the turtle. It is derived primarily from cells which advance peripherally from the geniculate ganglion along the great superficial petrosal nerve, but probably receives cells also which advance peripherally from the Gasserian ganglion along the maxillary nerve.

The relatively small submaxillary ganglion, in the chick, is genetically related to the mandibular nerve.

In embryos of the pig, as the writer has shown in an earlier paper,<sup>2</sup> the ciliary ganglion is genetically related to the oculomotor and the ophthalmic nerves, while the sphenopalatine, the otic, and the submaxillary ganglia are genetically related primarily to the maxillary and the mandibular divisions of the trigeminal nerve.

The results of a comparative study of the development of the cranial sympathetic ganglia in embryos of types of the several classes of vertebrates, as above set forth, warrant the conclusion that during the process of evolution the sources of the majority of the cells giving rise to the cranial sympathetic ganglia have become shifted cephalad. Whereas, in the lower vertebrates the cranial sympathetic ganglia are genetically related to the cervical sympathetics, the first spinal nerves, and the X, IX, VII, V, and III cranial nerves, the great majority of the cells taking part in the development of these ganglia in the mammals are derived from the Gasserian ganglion and the wall of the mid- and hind-brain via the oculomotor and the several divisions of the trigeminal nerves.

31. *The tract of Lissauer in the Rhesus monkey.* S. W. RANSOM, Northwestern University Medical School.

The observations which I shall report were made on pyridine-silver and Pal-Weigert preparations of the spinal cord of the Rhesus monkey.

In a Pal-Weigert preparation of the fifth cervical segment the tract of Lissauer is located in the apex of the columna posterior just lateral to

<sup>1</sup> Anat. Anz., Bd. 32, pp. 497-515.

<sup>2</sup> The development of the cranial sympathetic ganglia in the pig. Jour. Comp. Neur., vol. 23, pp. 71-96.

the entering fibers of the dorsal root. In comparison to the rest of the substantia alba the tract is lightly stained. It contains rather widely separated fine medullated fibers. Most of these fibers run longitudinally in the tract but some run across it from the dorsal root to the substantia gelatinosa Rolandi. Some medullated fibers enter the tract from the lateral portion of the dorsal root; but a study of the literature makes it clear that many of the medullated fibers in the tract are of endogenous origin.

In a pyridine-silver preparation of the seventh cervical segment the darkly stained tract of Lissauer fills the apex of the columna posterior and reaches from the substantia gelatinosa to the surface of the cord. An accumulation of subpial neuroglia is seen at the dorsal extremity. A neuroglia septum extends into the cord, separating the tract in question from the cerebellospinal fasciculus. The septum does not, however, reach the gray substance, and ventrally to it the tract of Lissauer spreads out into the lateral funiculus upon the lateral surface of the columna posterior. It goes over gradually into the fasciculus proprius of the lateral funiculus.

When one compares the number of medullated fibers seen in a Pal-Weigert preparation with the number of axons seen in pyridine-silver preparations it is obvious that the non-medullated fibers of this tract far outnumber those which are medullated.

The medullated fibers of an entering dorsal root pass over the tip of the tract into the cuneate fasciculus. The non-medullated fibers of the root separate out from among the medullated fibers before the root enters the cord and form a well defined lateral bundle. This lateral part of the entering root, consisting of non-medullated and a few fine medullated fibers, turns forward into the tract of Lissauer. This shows that the non-medullated fibers of the dorsal roots do not run with the medullated fibers into the fasciculus cuneatus but run in Lissauer's tract.

There is a very close relation between the tract and the substantia gelatinosa Rolandi. This substance contains many small nerve cells and non-medullated fibers. There is a constant interchange of fibers between it and the tract of Lissauer and everything points to it as the nucleus of reception of the non-medullated fibers of the tract and therefore also of the non-medullated fibers of the dorsal roots.

In conclusion it may be said that the tract of Lissauer in the monkey, like that in the cat, is composed chiefly of non-medullated fibers. These represent the intramedullary continuation of the non-medullated fibers of the dorsal roots and probably terminate in the substantia gelatinosa Rolandi.

Discussed by Sheldon, Hardesty and Huber.



32. *Chorionic ducts and intra-chorionic cysts in young human embryos.* FREDERIC T. LEWIS, Harvard Medical School.

33. *Further observations on the supports of the rectum.* T. WINGATE TODD, Western Reserve University, Cleveland, Ohio.

In a previous paper (*The anatomy of a case of carcinoma recti*, *Annals of Surgery*, 1913, vol. 59, pp. 831-837) the speaker indicated the clinical significance of the following structures: (1) The function of the fascia propria of Waldeyer (recto-sacral aponeurosis of J. W. Smith) in making the rectum a self-contained organ; (2) The function of the lateral ligaments (les ailerons) as supports of the rectum; (3) The fact that the lateral ligaments are mainly formed by the perineural tissue around the sacral nerves supplying the rectum, but also include the perivascular tissue around the middle haemorrhoidal vessels.

In the present communication the speaker first postulated that only the type of rectal prolapse which commences at the anal margin should be retained under the heading of 'prolapse,' the other varieties being in reality types of intussusception.

After a consideration of the various factors suggested clinically as causes of prolapse in infants, evidence was brought forward in support of the following contentions: (1) That the relative proportionate length and extensibility of the lateral ligaments in the infant at birth are approximately the same as in the adult. (2) That there is no 'laxity' of the lateral ligaments. (3) But that the rectum is already a pelvic organ at birth while the bladder and uterus lie at a higher level. (4) That in consequence of the relative low position of the rectum and of the fact that it is not shielded by an overhanging sacral promontory, the organ is in a position of greater mechanical disadvantage in infancy than in adult life. (5) Hence in infants if the pelvic diaphragm be weak, as in rachitis, there is every possibility of the occurrence of a temporary and limited procidentia of the rectum which does not require any operative measure for its treatment.

34. *On the occurrence of fat in the muscle fibers of the myocardium and of the atrio-ventricular system.* H. HAYS BULLARD, Anatomical Laboratories, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

In former papers ('12) I have presented data which indicate that the commonly accepted belief, to the effect, that microscopically visible fat does not occur in normal cardiac muscle fibers, has arisen largely from the fact that the technique for fat demonstration, as usually employed is inadequate to show the normal fat content of muscle fibers. It is only by using special methods upon fresh tissue that the full fat content is demonstrated. Fresh material is necessary, for fats are unstable compounds, as is now recognized by chemists but too frequently overlooked by histologists. For the demonstration of the fat content of muscle fibers and other tissues, Herxheimer's Scharlach R method offers marked advantages.

Bell ('11-'12) using the Herxheimer method, has shown in a remarkable series of feeding experiments that the 'liposome' content of skeletal mus-

cle and of cardiac muscle is influenced by the diet of the animal. He believes that some of the 'liposomes' are fat droplets, while others are fat mixed with some substance other than fat. He finds that starved rats show few 'liposomes' in the striated muscle, while those which have been on a diet rich in fat show a marked increase in the 'liposome' content of the muscle fibers.

Employing the Herxheimer method together with other methods, during the past several years, I have examined the cardiac muscle of more than a hundred animals for the presence of microscopically visible fat. In about forty hearts, the muscle fibers of the atrio-ventricular system were also examined. The animals included the mouse, rat, cat, dog, opossum, sheep, pig, ox, monkey and man. The results of this study, as here summarized, are later to be set forth in greater detail.

Normal cardiac muscle fibers of mammals contain a varying amount of fat in the form of droplets which react to fat stains and can be demonstrated microscopically. The droplets of fat are arranged in rows between the fibrillae or muscle columns and in the central peri-nuclear sarcoplasm. In size the droplets vary from 3 or 4 micra to the limit of microscopical vision. In some animals the fat is uniformly distributed among the muscle fibers, each containing approximately the same amount; on the other hand, there are often two general types of fibers, one type is heavily charged with fat, the other type containing little. The fibers which contain the large amount of fat correspond to the well known "dark fibers" of skeletal muscle, while those which contain little correspond to the light fibers.

During the later weeks of fetal life fat is normally present in the cardiac fibers (man, ox, pig, cat, dog). Earlier stages were not examined.

The normal fat content of cardiac muscle is not a product of degeneration, but is brought in to the fiber to serve as a source of energy and food. The quantity of fat in cardiac fibers is decreased in starvation and increased when the animal is kept on a fat diet.

Cardiac muscle fibers contain many granules which, after appropriate fixation, may be stained by the methods of Altmann, Benda, Weigert (modified) and Heidenhain. These granules are the true interstitial granules of Kölliker, bioblasts of Altmann, chondriosomes of Regaud, and *Q* granules of Holmgren. Granules of this type cannot be stained by fat stains and are not fat, although there is some evidence to show that they contain an albumino-lipoid.

Little fat is normally present in the muscle fibers of the nodal tissue of the heart or in the stem of the bundle of His, even when the myocardial fibers are crowded with fat. Typical Purkinje fibers of the sheep, pig and ox, contain a small quantity of fat but the amount is increased as the fibers take on cardiac character. More fat is present in the Purkinje fibers of species such as man, dog and cat, in which the Purkinje fibers are histologically similar to the cardiac type.

Literature cited: E. T. Bell, 1911, *Internat. Monatschrift f. Anat. u. Phys.*, Bd. 28, S. 297-347; 1912, *Journ. Path. and Bacter.*, vol. 17, pp.

147-158. Also H. Hays Bullard, 1912, Journ. Med. Research, vol. 27, pp. 55-65; 1912, Amer. Jour. Anat., vol. 14, pp. 1-46.

35. *Marchi technique: safer and easier clearing and mounting of sections.*

H. S. STEENSLAND, From the Pathological Laboratory of Syracuse University, Syracuse.

This communication is presented on the assumption that clearing Marchi sections in chloroform is generally regarded as the best technique at the present time. It is generally recommended that Marchi sections be cleared in chloroform and mounted in chloroform balsam because xylol and other clearing reagents, and xylol balsam, cause the black osmic acid staining of fat to fade. Clearing in chloroform, as recommended, presents certain wellknown difficulties in technique. These difficulties sometimes make the fate of valuable material uncertain, for example, material that has been obtained as a result of painstaking and time consuming experiment.

Obviously it would be a great advantage if Marchi sections could be cleared in oleum origani cretici. Most of the difficulties would be overcome. There would be no danger of drying and shriveling of the sections. The sections could be thoroughly blotted and flattened with smooth (not embossed) filter paper. The chloroform balsam could be carefully applied. The retraction of the balsam from a part of the space between the coverslips and the slides would be practically done away with. It would be possible to turn over valuable material to a technician for clearing and mounting with much less fear of loss of material and without placing undue responsibility and strain upon the technician. When a large amount of material is to be handled it could be handled with much less strain and exhaustion. Large sections would be more safely handled, which is of special importance when the tissues involved are cut into serial sections.

Ten years ago in the Pathological Laboratory of Syracuse University there were mounted a considerable number of Marchi sections. The technique made use of was the usual technique except that the sections were cleared in oleum origani cretici instead of chloroform. At the present time I have been unable to find any evidence of fading after comparison with control sections made from the original blocks of tissue. The control sections were cleared in chloroform and mounted in chloroform balsam according to the prevalent method.

In order to put the matter to a further test some of the newly cut sections were placed in a dish of oleum origani cretici. At intervals sections from the dish were mounted in chloroform balsam. No evidence of fading was found after ten days in the oil.

Oleum origani cretici is thus evidently a very much safer and easier clearing reagent than chloroform to use in the Marchi technique.



36. *Technical experiences:* (a) *Cataloguing lantern slides;* (b) *Permanent dry-mounts of the laryngeal cartilages;* (c) *The use of large tissue sections for demonstration purposes;* (d) *Degreasing bones.* G. L. STREETER.

(a) *Cataloguing lantern slides.* A fairly good print of a lantern slide may be obtained by laying the finished lantern slide directly on blue print paper and printing either in the sun or before an arc light. Such a print plainly shows the subject and character of any ordinary lantern slide. This fact may be utilized in cataloguing lantern slides. By printing ones whole collection in blue print paper and mounting these prints in a loose leaf note book it makes it possible to look them over quickly for ascertaining what slides are in the collection and their respective number. The writer prints six slides at one time in an 8 × 10 printing frame on uniformly cut sheets. These sheets are then assorted more or less according to subject. On each picture the slide number is written in and any other desired memoranda. The slides themselves are numbered and filed in the order of acquisition.

(b) *Mounting laryngeal cartilages.* A permanent and convenient mount of the laryngeal cartilages and adjacent rings of the trachea may be made by dehydrating the cleaned cartilages in alcohol, transferring them into xylol and then saturating them with melted parafin. Such cartilages are easily mounted on a base by means of metal adjustable standards and are durable enough to be trusted in the hands of students as demonstration specimens. The disadvantage of the usual dry preparation of the larynx lies in its tendency to shrink. This is largely avoided by the above method in which the natural moisture of the specimen is replaced by parafin. In the process of dehydration there is some danger of warping the cartilages, but this can be prevented by cutting out little wooden forms to which the cartilages are tightly laced with thread, or by fitting them over or between tubular bottles of different sizes. They should be kept on these until they come out of the melted parafin. In the six preparations we have made at Ann Arbor we included the hyoid bone. The preparations show an interesting variation in the form of the cartilages and in the character of their ossification, so much so as to warrant the increase of our collection for the study of these particular features.

(c) *Demonstration sections.* We have found in our laboratory that large stained celloidin sections mounted and projected like lantern slides form an excellent means of demonstrating certain features in regional and macroscopic anatomy. It may be suggested that where successful preparations of this kind are obtained there would be an advantage in cutting extra sections and saving them as duplicates to be exchanged for similar preparations from other laboratories. The following sections, which had been prepared in the above manner, were demonstrated with the lantern: (a) Transverse section through top of adult head showing layers of scalp, formation of cranial vault, dura, falx cerebri, superior longitudinal sinus, together with the brain and the membranes directly covering it; (b) Cusp of adult semilunar valve,

flattened out to show distribution of connective tissue and formation of lunulae; (c) Transverse section through adult cavernous sinus; (d) Frontal section through adult larynx showing false cords cut transversally and vocal lips together with cartilages and musculature; (e) Sagittal section through the adult temporomandibular joint; (f) Transverse section of adult penis; (g) Transverse section through neck of new-born babe showing especially the distribution of fascia; (h) Sagittal section of finger of newborn babe showing metacarpal and three phalangeal bones with epiphyses, joints and muscle attachments.

(d) *Degreasing bones.* The bones of the extremities from fatty subjects may still have after maceration a large amount of fat in them. Most of this may be easily, economically and safely removed by placing them in a drying oven and sweating the fat out. A fatty femur after being in an oven at 110° C. for two hours loses 25 per cent in weight by removal of the fat. On coming from the oven the bones are briefly rinsed in cold gasoline and are then bleached in potassium permanganate followed by sulphurous acid. They are then ready for study. We have found that we can increase the durability of fragile bones by immersing them in melted paraffin. This darkens the bone but does not interfere with its suitability for student use.

37. *On the nature of fat cells.* H. G. WEISKOTTEN AND H. S. STEENSLAND, From the Pathological Laboratory of Syracuse University, Syracuse.

When rabbits receive intravenous injections of maximal sublethal doses of saponin large territories of the marrow undergo necrosis. The parenchymal cells and fat cells soon undergo autolysis leaving mainly the free fat droplets, originally contained in the fat cells, to represent the original architecture of the marrow. The fat droplets appear to remain largely in the original loci of the fat cells, in which they were contained.

Subsequently there occurs apparently complete regeneration of the marrow with the restoration of the original proportions of parenchymal cells and fat cells. By examination of sections of the marrow from animals at various stages after injection it appears to be possible to determine the manner in which the fat cells are regenerated and to determine the nature of these fat cells.

To a large extent the necrotic tissue is invaded first by free endothelial cells. These cells tend to arrange themselves at the periphery of individual fat droplets and to fuse together into multinucleated cytoplasmic masses, which envelop the fat droplets. These masses constitute the wellknown foreign body giant cells. Subsequently a large part of the cytoplasm of these cells disappears, the nuclei decrease in number and the cytoplasm becomes reduced to the thin envelope characteristic of the ordinary fat cell. In this envelope there persist one or more nuclei, which become crescentic as the cytoplasmic layer becomes thin, like the nucleus of the ordinary fat cell. The disappearance of nuclei evidently is brought about by a process of nuclear resorption or karyolysis.

One possibility that arises is that the fat cells originally do not become completely necrotic before the contained fat droplets become surrounded



by foreign body giant cells; that the foreign body giant cells disappear and there remain the original fat cells.

In the manner described the fat cells are regenerated to a large extent before the parenchymal marrow cells are regenerated. Mainly after the fat cells are restored the hemoblastic centers develop in the corresponding regions and the marrow is completely regenerated.

The concept that we wish to present, on the basis of what has been described, is that fat cells are endothelial cells containing what may be called, by way of comparison, a physiological foreign body, namely fat. Ordinarily, in our experience, fat cells are regarded as mesenchymal cells or fibroblasts or connective tissue cells in whose cytoplasm fat has been stored up.

In general, in many forms of lesion free fat occurs and is taken up by foreign body giant cells. This may be in situations in which fat cells are not normally present. In such cases the foreign body giant cells do not survive in the locus as fat cells, perhaps because fat cells do not normally exist in the locus.

That foreign body giant cells are formed as a result of the fusion of endothelial cells has been shown by the work of Mallory and his pupils.

38. *The development of the septum atriorum.* ROBERT RETZER, University of Chicago.

39a. *The passage of the ovum through the uterine epithelium in Geomys bursarius, with demonstration of wax reconstructions.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

Within the order of Rodentia there exists a greater variety in the implantation of ovum and formation of decidual cavity than in any of the other main divisions of the Mammalia. The range of variation extends from those forms, like the rabbit, in which the whole of the uterine lumen is utilized, to that of mouse and rat, where only a restricted portion of the uterine cavity is transformed into a decidual cavity. Then follows those peculiar forms, as *Citellus* (spermophilus), in which the writer in 1902 and 1903 described for the first time a rodent in which the trophoblastic layer of cells at close of segmentation caused the destruction of a small area of the uterine epithelium at the antimesometrial portion of uterine cavity, followed by an outgrowth of trophoblastic cells into the mucosa to form a nutrient organ, followed by the atrophy and disappearance of this organ upon the completion of the allantoic placenta at the mesometrial portion of the uterus; the whole of the uterine cavity being utilized for a decidual cavity, as in the rabbit. And lastly are found those forms in which, as in man, the ovum passes entirely through the uterine epithelium and a new decidual cavity is formed in the mucosa and independent of the uterine lumen. The first rodent of this type to be described was the guinea-pig, so beautifully worked out by Graf Spee. In *Geomys* we find a second rodent of this type, but with the following important points of difference from the guinea-pig which are shown in these reconstructions. In the guinea-pig the ovum passes



through the uterine epithelium at close of segmentation, and while it is very small in volume, the small perforation is quickly closed and the uterine cavity completely separated by epithelium from the new decidual cavity. In *Geomys*, on the contrary, the ovum perforates the uterine epithelium in the blastula stage, and when of so large a diameter that the edges of the large rounded perforation of the uterine epithelium cannot grow together as in the guinea-pig, or be filled with a fibrin plug as in man, this opening persists during the entire preplacental period. The epithelium at the lip of perforation is somewhat everted and gives a point of attachment to a zone of the trophoblastic layer of the blastocyst. The dorsal portion of the trophoblastic layer extends across the opening, and by this zonal attachment to the epithelial lip completely shuts off the uterine lumen from the new decidual cavity. The cells of the mucosa are broken down and the decidual cavity is rapidly enlarged. The blastocyst sinks down into this cavity but continues to be suspended by the above described zonal attachment to the lip of the epithelial perforation. This zone will ultimately form the outer margin of the allantoic placenta. A detailed description of the unique preplacental development in *Geomys* with plates will be published in the near future.

39b. *An improved electric microscope lamp, with demonstration.* THOMAS G. LEE, Institute of Anatomy, University of Minnesota.

This lamp was devised by the writer to meet the demand for a small compact and portable lamp for individual staff or student use in the Minnesota Institute of Anatomy, and has proved to be so satisfactory that it is here demonstrated for the benefit of the members of the Association. The lamp consists of a small vulcanite base fitted with a silvered reflector and a socket into which can be screwed as desired either a 2, 4 or 6 candle-power Mazda lamp with miniature base; a metal cap supported by 3 rods which fit into the base shuts off all side light and gives the necessary ventilation. In the top of this cap is an opening the same size as the base of the Abbe condensor. A device on the top of the metal cap holds in place over this opening the ordinary blue or ground glass plates commonly used with the Abbe condensor, thus giving at all times monochromatic light of uniform intensity. The base is fitted with flexible electric fixture wire terminating in a small plug which fits into a socket in the table top. The entire lamp is small, about two inches in diameter and in height, so that it readily fits in under the Abbe condensor when the mirror is pushed to one side. When not in use, the lamp can be put away in the student's locker with the rest of his outfit. A stock automobile Mazda bulb of standard make of 6 volts, 5 watts, alternating current is used in this lamp. It is the smallest available, the least expensive, and will withstand rough usage. In fitting up the laboratory tables a step down transformer is connected with the feed wire. This reduces the current used from 110 volts to 6 volts. These transformers are small, inexpensive, and can be had of any capacity desired, are used in the trade in sign-lighting apparatus. The wires leading from trans-

former are run underneath the tables and connected to the sockets which are set into table top at any desired point. A detailed description of this apparatus with illustrations will appear in the near future.

40. *The growth of organs in the albino rat as effected by gonadectomy.*

S. HATAI, The Wistar Institute of Anatomy.

1. Except in the remaining sex gland itself, the partial removal of the sex glands does not produce any significant alterations in any of the ductless glands aside from a general tendency to a slight increase. Apparently this increase in the remaining gland is sufficient to compensate for the functions of the lost gland.

2. The total removal of sex glands, however, induces alterations in all the other glands, particularly in the thymus and hypophysis. The suprarenal glands show opposite reactions in the two sexes. In the case of the males, the suprarenal glands show an increase of 15 per cent, while in the female there is a 20 per cent reduction.

3. The total removal of the sex glands tends to increase the resemblance between the two sexes, or in other words, to reduce the differences in those secondary characters which, in the normal animal, are modified according to sex.

41. *Ganglion cells of the terminalis nerve in the dogfish.* PAUL S. McKIBBEN, The Western University, Ontario, Canada.

42. *The fate of the ultimo branchial body in the thyroid.* B. F. KINGSBURY, Cornell University.

43. *The position of the normal stomach, with observations on the movements of the diaphragm.* BURTON D. MYERS, Indiana University School of Medicine.

Forty young adults, nineteen to twenty-six years of age, twenty-eight men and twelve young women, were given a buttermilk-barium sulphate meal. Their stomachs were then examined fluoroscopically.

Prior to giving the meal, copper washers were fixed with adhesive tape to the abdominal wall in the mesial sagittal plane, one on the processus xyphoideus, a second at the transpyloric plane, a third on the umbilicus, and in the young women, a fourth was placed at the intersection of the intertubercular plane and the linea alba. These washers, plainly visible on the fluoroscopic screen, give the chief horizontal planes and the mid-sagittal plane. An adjustable diaphragm made it possible to cut the rays to a vertical slit, to a horizontal slit, or to a very small square opening, in which latter case, the rays are nearly parallel.

Inasmuch as in the young women, only a thin kimona intervened between the washers and the thirteen inch-square fluoroscopic screen, while in the case of the men, the screen was placed immediately upon the washers and abdominal wall, the error due to divergent rays is negligible, and checked by narrowing the diaphragm.

Sheets of very thin tracing paper were placed upon the fluoroscopic screen and tracings made of the stomach while filling, when full, in deep-

est inspiration and fullest expiration accompanied by contraction of abdominal walls. Tracings were also made of the diaphragm, showing its normal position, its swing in normal respiration, and its extreme positions in forced inspiration and expiration. These same tracings were repeated with the individual in horizontal position (on back). X-ray photographs were made of five cases, for comparison and check.

In males, when standing, the average position of the lower border of the stomach was found to be one inch below the umbilicus, the extremes being from one inch above to three inches below this plane. In females, when standing, the lower border of the stomach was found to be three inches below the umbilical plane, the extremes being one and three-eighths to four and one-half inches below the umbilical plane. When standing, the stomach is either J- or cow-horn shaped. The pyloric valve points upward, backward, and to the right. When lying down, the pyloric valve is one-third of an inch below the transpyloric plane, and in  $12\frac{1}{2}$  per cent of cases, it points upward, backward, and to the left; the descending portion of the duodenum then lies posterior to the pyloric portion of the stomach.

The cardiac stomach is not a storehouse for food, as commonly stated, but when standing, a gas pocket. The stomach fills from above downward, the upper border of its contents remaining, during filling, at the level of the esophageal opening.

The stomach is always as big as its contents. Its shape depends upon the quantity of its contents, the position of the body, the distention of adjacent viscera, peristalsis, and respiration. In certain cases even the beat of the heart gives a blow to the stomach wall which causes a wave to run across the surface of its contents.

The stomach is normally in a state of tonic contraction so that when one lies down, the portion of the stomach over the vertebral column tends to empty and contract while the fundic portion accommodates an increased portion of the stomach contents.

In the erect position, the fundic portion of the stomach looks upward, not backward as stated by His and Cunningham. The surfaces are not up and down, but anterior and posterior, or antero-superior and postero-inferior as we stand or lie down. The greater curvature is not higher but lower than the lesser. The lesser curvature does not become convex when the stomach is filled, filling being accommodated by distention of the greater curvature. The position of the incisura angularis, with reference to the pyloric valve, varies with the high or low position of the pyloric valve.

The normal position of the diaphragm is higher when one is in the horizontal, than when in the erect position. Not infrequently, contraction of the abdominal wall is accompanied by descent of the diaphragm. Though some females employ costal respiration almost entirely, as do some men, others show as great a swing of the diaphragm in normal respiration and as great extremes of movement of diaphragm in forced inspiration and expiration as is found in men.



44. *The form of the stomach in mammalian embryos.* CHESTER H. HEUSER, The Wistar Institute of Anatomy.

In *The American Journal of Anatomy* ('12, vol. 13, pp. 477-503), F. T. Lewis described the form of the stomach in young human embryos and the development of its primary subdivisions. During the past year, with the aid of Bullard Fellowships awarded at the Harvard Medical School, the writer has made a similar study of the embryonic stomachs of the albino rat, pig and sheep, and the work is approaching completion. In all of these animals as in man, the epithelial stomach is <sup>early</sup> nearly subdivided into cardiac and pyloric portions, separated by the angular incisure. The pyloric portion is often only obscurely subdivided into an antrum and vestibule, but the cardiac portion clearly presents a corpus and fundus. The early development of the gastric canal is a notable feature in all the animals studied, as could be seen in the series of wax reconstructions which were presented as a demonstration.

45. *On the phylogenesis of the heart.* A. G. POHLMAN, St. Louis University.

The first sign of a division in the heart occurs in the lung-fish with the appearance of an incomplete auricular septum. The next step is found in the amphibian with an incomplete division of the bulbus by the spiral valve (see below) in addition to the incomplete auricular septum. The third stage is found in the reptile with the complete separation into two auricles and the completed division of the bulbus and the appearance of an interventricular septum. The fourth stage comes in the crocodile family with complete division of the heart into four chambers, the right side having greater capacity than the left. The fifth in the bird and mammal with a four-chambered heart and equal capacity on the two sides. Just as the foramen ovale appears as a functional compensation for the inequality of return to the two sides of the heart in the fetal bird and mammal, so the foramen of Panizza may be a functional adaptation to the unequal quantities of blood expelled by the two ventricles. This latter point is not thoroughly understood. There is no proof of a segregation of arterial and venous blood throughout the vertebrate scale excepting in the postfetal bird and mammal and possibly in the crocodile family.

Demonstration of reconstruction of the bulbus cordis in the frog.

This reconstruction shows: (1) that the spiral valve cannot function in the manner described as a means of separating the bulbus into an aortic and pulmo-cutaneous compartment; (2) that the carotid arteries arise by a common opening; (3) that this opening is again in common with that of the right aortic arch; (4) that the left aortic arch has an entirely independent opening about at the level of the pulmo-cutaneous opening and is distinct from the right aorta. Both left aortic and pulmo-cutaneous openings have a valve which is wanting in the right aortic opening; (5) that the spiral valve may be interpreted as an incomplete division of the bulbus analogous to the incomplete division of the auricle

to page 130.

# ERRATUM

The Anatomical Record, volume 8, number 2, February, 1914, Abstract 44, Chester H. Heuser, page 130, line 9, for *nearly* read *early*.





and the relations of the left aortic arch and pulmo-cutaneous artery are suggestive of a phylogenetic step completed in the turtle.

1. Demonstration of the canalis cranio-pharyngeus in the rabbit showing the possibilities in experimental work in this form of determining the relation of naso-pharyngeal irritations upon the hypophysis and upon the pharyngeal hypophysis and its bearing on the adenoid question. Preparation of Dr. Eugene Senseney.

2. Demonstration of elastic ligaments in the middle ear region of the chicken which may afford resistance to the pull of the tensor tympani. Preparation by Mr. Wilson and Mr. Shores.

3. Trilocular heart with pulmonary stenosis in a child of ten years (case of Dr. Ralph Thompson) to show transposition of vessels and an absolute lack in separation of arterial from venous circulation.

46. *Some notes on early twin human embryos.* JAMES CRAWFORD WATT, Anatomical Laboratory of the University of Toronto.

These twin embryos are Nos. V and VI in Prof. J. Playfair McMurich's collection. They are respectively 2.75 mm. and 3.35 mm. in length. They are almost identical in development, but one has a very deep concave dorsal bend, while the other is almost flat. Embryo V has 17 to 18 paired somites, Embryo VI has 18 to 19. They thus serve to fill in an interval hitherto lacking in good specimens.

In the alimentary system the buccopharyngeal membrane is just rupturing, the pharynx has three gill-pouches and a medium thyroid depression. Connection with the yolk sac is very extensive. The cloaca is small but is divided into rectal and bladder bays.

The notochord is still attached to the gut throughout much of its extent. In places it shows the remains of a notochordal canal and a chord of cells extending from the medullary plate to the notochord in both embryos and also from the notochord to the cloaca in Embryo V. represents the neurenteric canal. These are the oldest embryos recorded which exhibit these remains.

The urinogenital system of Embryo VI consists of five pronephric tubules on each side, united to the Wolffian duct. Behind are ten to eleven mesonephric vesicles on each side, not united to the duct, which extends from the ninth to the sixteenth mesodermic segments. In Embryo V the duct on the left extends from the ninth to the sixteenth segment and receives seven pronephric tubules, and on the right it extends from the seventh to the sixteenth segment and receives eleven tubules. There are five to seven mesonephric vesicles behind these. Many of the tubules exhibit nephrostomes and there is an external glomerulus in the ninth segment of Embryo VI. There is evidence of dysmetamerism, as many tubules occur in pairs in the segments on each side.

The heart is a simple S-shaped tube. Only the large embryological vascular trunks are developed, including two branchial arch vessels on each side. The brain shows three primary vesicles, optic vesicles, hypophysis, and seven neuromeres in the hind brain with four ganglia—tri-

geminal, acusticofacial, glossopharyngeal and vagus—attached to definite neuromeres. A complete and full description of the embryos will be published shortly.

47. *Notes on the skull of a human fetus of 50 mm.* C. C. MACKLIN, Anatomical Laboratory of the University of Toronto.

The skull described occupies a position intermediate between the 28-mm. stage of Levi and the 80-mm. stage of Hertwig, and is interesting in that it shows indications of a reduction of the lateral walls of the chondrocranium in the form of small isolated remnants of cartilage situated dorsally and laterally. Other similar isolated cartilages also occur, among which may be mentioned a rudiment of the aliochlear commissure; an ephemeral representative of a primitive nasal concha, seen in the middle meatus of the nasal cavity; and a small mass in the orbit, lying against the nasal capsule. A minute additional paraseptal cartilage is noted, attached to the nasal septum, and related to the vomerine anlage, while the anterior paraseptal cartilage presents an interesting transitional stage in which it may be directly compared with that of such form as the rabbit, and the paraethmoidal cartilage, related to the lacrimal bone and duct, is also well developed.

The condition of the structures surrounding the foramen magnum throws some light on the development of this region, especially in regard to the part played by the occipital vertebra, which is, in this stage, rather distinctly outlined. A detailed description of the skull, with figures showing its reconstruction, will shortly be published.

48. *The development of the pancreas in selachians.* RICHARD E. SCAMMON, Institute of Anatomy, University of Minnesota.

The pancreas of selachians is remarkable in that it arises from a single diverticulum which has generally been described as dorsal in position. It is, however, very difficult to determine the exact position of this anlage by the customary means of reconstruction. For this purpose, therefore, I have applied the method of graphic reconstruction devised by Weber. This method, which seems to me a most valuable device for studying the early stages of the larger glands, has apparently been employed only by its originator some ten years ago in an extensive study of the earliest stages of development of the liver and pancreas in Amniotes. The details of this procedure can hardly be given in the space allotted here. They may be found in Weber's original work (*Arch. D'Anat. microsc.*, T. 5, '03), and will be again presented in this paper in its final form. In general terms, it is a modification of the graphic method of reconstruction in which the varying thickness of the epithelium of the reconstructed archenteron is represented in corresponding varying shades of color so that one may study the various areas of epithelial thickening in much the same way that one interprets the elevations of land in a topographic map.

A reconstruction made in this manner of the midgut of an *Acanthias* embryo 5.2 mm. in length, which is of a stage well preceding any indica-

tion of the pancreas as an outpouching, shows in the future pancreatic region of thickened epithelium which includes not only the dorsal zone of the gut but extends well ventrally. This pancreatic area is connected with a large thickening ventral and anterior to it which constitutes the liver anlage. These two thickenings, the pancreatic and hepatic, constitute, with the thickened epithelium connecting them, a ring which passes obliquely completely around the archenteron. The existence of such a pancreatic-hepatic ring was postulated long ago by Brachet and has been demonstrated to a great extent in Amniotes by Weber.

Reconstructions by Weber's method of the midgut regions of embryos of *Acanthias* 7.5, 9 and 10 mm. in length, show a gradual breaking up of this ventral hepatic segment. Between the two the epithelium remains somewhat thickened and in this occurs a particularly thickened patch which occupied the same position as does the anlage of the ventral pancreas in the higher vertebrates. This thickened plate never produces an outpouching and disappears in older embryos. While the dorsal outpouching of the pancreas is a single one, there is in *Acanthias* at least, an early indication of division into right and left lobes. This division is not very distinct and can hardly be demonstrated without Weberian and plastic reconstructions. In *Acanthias* the left lobe lies anterior to the right and is the smaller. It forms in embryos from 15 to 35 mm. in length a distinct antero-ventral mass which later is to a considerable extent incorporated in the descending limb of the gland. In such other selachians as I have studied this lobe lies posterior to the right one. This is probably because the left lobe is not differentiated until the gland begins to separate from the gut and in all selachians except *Acanthias* separation takes place antero-posteriorly. I do not think that the bilobed dorsal pancreas is to be regarded as a primitive condition. Rather it is produced by the clockwise rotation of the gut and the formation of the spiral valve. Bilobed dorsal pancreas anlagen are found in mammals and in selachians but have not been clearly demonstrated elsewhere unless one accepts the observations of von Küppfer the value of which has been rendered doubtful by the work of Piper and Nicolas. It seems then that the bilobed form of dorsal pancreas is limited to those forms in which the clockwise rotation of the gut (common to some extent to all vertebrates) takes place at an early period and that this form of pancreas is due to that rotation.

A more complete statement of the development of the selachian pancreas will be published in the near future.

*49. The development of the gall bladder and bile ducts in amblystoma.*

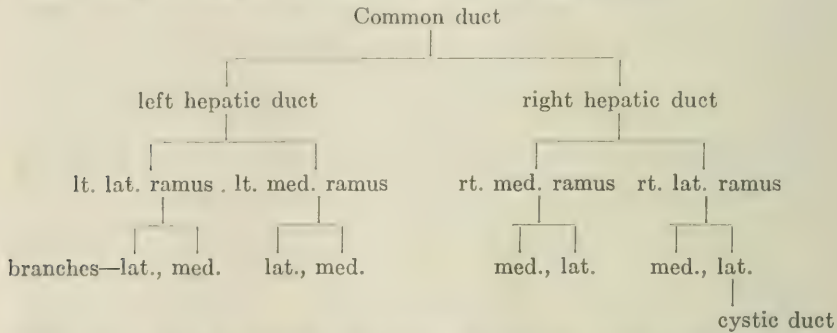
E. A. BAUMGARTNER, Institute of Anatomy, University of Minnesota.

Models of embryos 4.5 mm. long show that the liver anlage is a ventral, somewhat caudal projection of the gut lumen caudal to the heart. That this does not correspond to the caudal hepatic duct described in chick is shown by later stages. In models of embryos about 7 mm. long we see this early ventral outpouching has turned cranialward. In the ventral wall where the primitive common duct joins the gut lumen a me-



dian depression shows the earliest anlage of the gall bladder. In stages of about 9 mm. length the folds have become numerous and are the anlage of radicles of the main hepatic ducts. That they are not entirely formed by the tunneling in of blood vessels as has been described by Shore, is shown by the fact that furrows are found in which no blood vessels are present. The early anterior, and laterally extending duct shows beginning constriction and elongation into right and left hepatic ducts. A little later the gall bladder has shifted to the right and is no longer widest transversely. The cystic duct also has shifted to the right, now being attached to the ventral side of the right hepatic duct. The shifting of the gall bladder is accompanied by a shifting of the entire caudal part of the liver due to the sinistral and ventral growth of the stomach and duodenum.

From graphic reconstructions of late embryos we see an arrangement of the biliary apparatus as shown in the following outline:



Occasionally the left medial and right medial rami join to form one duct which subdivides as a single stem. This is a secondary union and persists in the adult. Also in some cases the cystic duct was found to be one subdivision further removed, that is, it was attached to a radicle of the lateral branch of the right hepatic ramus.

In embryos between 10 and 12 mm. in length, division and growth of the early duct into right and left hepatic ducts and of these into rami takes place. A graphic reconstruction of a 13 mm. stage shows that this has taken place. The gall bladder here is now longest postero-anteriorly and the short cystic duct extends upward and to the left. A model of a 14 mm. embryo shows that the gall bladder has shifted more to the right and that its duct which is attached to the extreme anterior end extends somewhat upward and to the left.

A model of the biliary apparatus of a slightly older embryo shows greater development of the duct system. The gall bladder is longer anteroposteriorly, its duct is attached more caudally and is almost horizontal. In a 15 mm. embryo there is a short common duct, but the right and left hepatic ducts are longer. The right lateral shifting here is more marked. The cystic duct extends horizontally to the left. This stage marks the extreme caudal attachment of the cystic duct to the gall bladder.

At the 20 mm. stage the lateralward shifting is very marked. The right hepatic duct is now somewhat dorsal to the left. Here the right and left medial rami have united to form one duct. The gall bladder has increased very materially in size, particularly in its caudo-cranial diameter. The cystic duct has shifted toward the anterior end and now projects to the left and downward. The extreme of the lateralward shifting has been reached in a 35 mm. embryo. The right hepatic duct here is quite dorsal in position in relation to the left. Its lateral radicles are also more dorsal than its medial. The left lateral ramus has turned caudalward to supply hepatic radicles to the further caudal extending left lobe of the liver. The gall bladder has increased in its dorso-ventral diameter. Its duct is attached at the anterior end and extends toward the left and downward as before. In a 45 mm. embryo and larger ones the right and left hepatic ducts are again more nearly in a horizontal plane. The cystic duct is attached to the anterior medial end of the gall bladder.

*Summary.* (1) The ductus choledochus develops as the early anterior directed duct from the gut. (2) The right and left hepatic ducts develop as divisions of the ductus choledochus and by growth and division form the hepatic rami and branches. (3) The gall bladder begins as a median ventral outpouching of the early anterior forming liver anlage. It is first widest laterally and finally obliquely caudo-cranially. (4) There is an early right lateral shifting of the biliary apparatus. Along with this there is a constant shifting direction of the cystic duct in keeping with the dorsalward migration of the gall bladder. (5) The cystic duct is early closed off with the right hepatic and finally is attached to the lateral branch of the right lateral ramus.

50. *Heteroplastic development of eosinophil leucocytes and of hematogenous mast cells in bone marrow of guinea-pig.* HAL DOWNEY, University of Minnesota.

According to Weidenreich and many others the granules of eosinophil leucocytes are composed of exogenous substance derived from hemoglobin or its dissociation products. Weidenreich's conclusions are based on a study of local development of eosinophils in hemolymph nodes, and in the taches laiteuses of the omentum of rabbit following the injection of guinea pig erythrocytes into the body cavity. Weidenreich's conclusions are based on very good evidence, as the writer can testify from personal study of his material. However, for the bone marrow the evidence for Weidenreich's view of the origin of the eosinophils is not so conclusive. The main facts in its favor are the observations of Marwedel and others that there is considerable degeneration of erythrocytes in the bone marrow, especially during inflammation, when the process is accompanied by the formation of numerous pigment cells and the occurrence of numerous eosinophil myelocytes which contain pigment granules.

The other view in regard to the origin of eosinophil leucocytes, especially in the bone marrow, is, that the granules of these cells are true

endogenous plasma differentiations of the non-granular cells concerned, and that the cells and their granules undergo a definite evolution during their differentiation which is accompanied by changes in the staining reaction of the granules. The granules are basophilic when they are first formed, but they gradually ripen into acidophilic granules. Such a process has been described in more or less detail by Ehrlich, Hirschfeld, Pappenheim, Maximow and others. Weidenreich admits the presence of basophilic granules in eosinophil myelocytes, but states that they are either endogenous granules which have nothing whatever to do with the eosinophil granules, or that they are fragments of the nucleus which are of the same size as the granules and therefore difficult to distinguish from them. He believes that the eosinophil granules are the expression of the taking-in of substances and particles which are formed by a special kind of erythrocyte degeneration either without or within the cell. He states that no other form of the development of the granulation has ever been observed, especially not a gradual differentiation from a non-granular protoplasmic cell-body, as is known for the special myelocytes.

Our problem is to determine whether these statements of Weidenreich are really true for the bone marrow, or whether the view stated above is the correct one. Without figures a detailed description of these investigations is impossible, however, the results can be stated in a few words and the demonstration of the slides will make the drift of the investigation clear.

The preparations show clearly that the granules of the eosinophil myelocytes are gradually differentiated out of a non-granular protoplasm, and that the first granules are basophilic. The preparations also show clearly that these basophilic granules are transformed directly into the eosinophilic granules. Both of these statements are denied by Weidenreich.

The first granules are small and not numerous, and it is very often difficult or impossible to distinguish them from the myelocytes of the special cells, a point which has already been emphasized by Maximow, who believes that in embryo rabbits and guinea pigs there is a common granular parent-form for both eosinophils and special cells. Fortunately it frequently happens that some of the granules enlarge far beyond the size of the special granules while there are still only a few of them in the cell. This fact enables us to detect some of the very early stages in the differentiation of the eosinophils and to distinguish them from the special cells. The granules remain unequal in size during the differentiation of the cells, the youngest granules being small and basophilic, while the older ones are larger and eosinophilic. Some of the granules change their staining reactions while they are still small, while others remain basophilic until they have reached a size even greater than that of the fully differentiated granule before such change takes place. That these larger granules do not disappear, and that they are transformed directly into the eosinophil granules is shown by the fact that many of the largest ones are stained in the acid component of the staining mixture, while others are of a mixed tone. These developing granules are round, and



variable in their staining reaction and in their size, but later they are of about equal size, which is less than that of the largest developing granules, and they become elongated and uniformly acidophilic. In other words, the granules of the eosinophil leucocytes during heteroplastic development are gradually differentiated out of a non-granular protoplasm, and they pass through a gradual progressive evolution which is the equivalent of that of the special granules, and which indicates that they are endogenous formations. If hemoglobin or its products has anything to do with their formation it is not evident from a study of the normal bone marrow with the ordinary histological methods.

The fact that the young granules of both eosinophil and special myelocytes of rabbit and guinea pig are basophilic has led Pappenheim and his students Benacchio, Kardos and Szécsi to the conclusion that there are no true mast leucocytes in the blood of these animals, at least that no such cells are formed in the bone marrow. They concluded that the mast cells are merely unripe eosinophils and special cells. An investigation of this question had been completed and the figures drawn when Maximow's paper dealing with the same subject appeared in the last number of the *Arch. f. mikr. Anat.*, consequently there is little left for the writer besides confirmation of Maximow's results. In so far as the guinea-pig is concerned, there is no difficulty whatever in recognizing the mast granules the moment they appear. The mast leucocytes of this animal are so definite and characteristic that they can be identified from the moment of their first appearance in the bone marrow. The slides which have been demonstrated give sufficient proof of this fact, so it is unnecessary to give further detail, excepting the general statement that the staining reactions of the mast granules and their general shape and size are very different from those of the young eosinophil or special granules. Therefore, for the guinea-pig, the results obtained by Pappenheim and his students from their investigation of this question are herewith most emphatically rejected.

The results of the present investigation show further, that heteroplastic development of various types of granulocytes from non-granular cells is a very active process in the marrow of adult guinea-pig. According to Helly the only normal process of leucocyte regeneration in adult human bone marrow is a homoplastic form of development by mitosis of the corresponding granular myelocytes. All non-granular cells of the marrow are, according to this author, either erythrogonia which are associated with anemic degeneration, or pathologically "entdifferentiated" myelocytes.

Many mitotic figures are seen in the various types of myelocytes of the guinea-pig marrow, which shows that active mitosis continues for some time after the cells have differentiated granules; but besides this homoplastic form of development, which is the only one recognized by Helly, there is also very active heteroplastic regeneration from non-granular cells.

51. *Some variations of the thoracic duct.* HENRY K. DAVIS, Department of Anatomy, Cornell University, Ithaca.

TUESDAY, DECEMBER 30, 2.00 P.M. TO 5.30 P.M. THE FOLLOWING DEMONSTRATIONS WERE PRESENTED:

1. *The Frankfurt method of mounting microscopic sections in photographic gelatine, without cover-glasses.* WILLIAM H. F. ADDISON, University of Pennsylvania.

Specimens of thin sections of human cerebrum were shown covered with photographic gelatine instead of Canada balsam and glass. For more than twenty years Professor Edinger of the Neurological Institute in Frankfurt has been trying such things as varnish, celluloid, celluloid films and kodak films as an inexpensive and convenient substitute for the usual mounting and covering substances, and at last suggested to Dr. R. E. Liesegang, the well-known photographic chemist who was working in the laboratory, the use of gelatine. The method has been in use for several years, and is especially convenient with large sections of the brain stained by the Weigert method. It may, however, also be used on celloidin, paraffin or frozen sections, and with any stain except aniline dyes in aqueous solution. The photographic gelatine is soaked in water until soft (1 part of gelatine in 10 parts of water for about an hour) and dissolved by gentle heat, by immersing the wide-mouthed bottle containing the softened gelatine in heated water (not over 50°C). The gelatine solution is filtered through filter paper at 39°C. in an oven, before using. The sections must come into water before they are ready to mount. After draining off the superfluous water from the preparations on the slide, the gelatine solution is poured over the slide, and the slide placed in a dust-free atmosphere to dry. A gentle current of air aids in the hardening process. The result in several hours to overnight is a very thin hard covering of gelatine, which appears perfectly transparent. It is best to prepare the gelatine solution in small quantity, for example, 50 or 100 cc. and to make it fresh each time before using.

The method was first published by Liesegang in 1910 (*Zeitschrift für Wissensch. Mikr. Technik*, Bd. 27) and further developments of it have been described recently by Professor Edinger (*Neurologisches Centralblatt*, Juli, 1913) where a description is given of further special details. Thus in dealing with thick sections it is well to soak them in the 10 per cent gelatine solution in the oven at 39°C. for an hour before mounting, to insure penetration of the gelatine. This is not needed with small ordinary sections. The hardening process after mounting may be assisted by dipping the slides in 10 per cent formalin about an hour after pouring on the gelatine.

Another special use is to cover preparations which have been stained for fat by Sudan III, or Scharlach R. where a dehydration with alcohols must be avoided. The sections are mounted with gelatine immediately from water with no other after-treatment. Specimens of heart muscle mounted in this way retain the stain unimpaired.

The method is still being perfected but already it has proved inexpensive and convenient for large brain sections and useful in the preservation of sections stained for fat, as well as for other more ordinary preparations.

2. *Preparations to show the formation of red blood-cells in the developing thymus of the pig.* J. A. BADERTSCHER, Cornell University.

3. *Demonstration of preparations showing the behavior of endothelium after the introduction of emboli in the portal vein.* ROGER P. BATCHELOR, Johns Hopkins University.

The experiments of Evans and co-workers have conclusively demonstrated the endothelial origin of giant cells in liver after infection with a suspension of tubercle bacilli. The endothelial nature of the cells concerned was established by means of vital stains. The study here demonstrated was suggested by Dr. Evans and undertaken to test whether this phenomenon was peculiar to the tubercle bacillus or any micro-organisms and secondly to study the reaction of the endothelium of relatively large branches of the portal tree. A preliminary injection of trypan blue was made and a week later, the colic vein exposed and five cubic centimeters of an aqueous suspension of baked egg albumen injected toward the liver.

The albumen flakes which were very minute and easy to cut in serial sections of the tissue subsequently, were made by beating the albumen to a fine foam and baking the same at high temperature.

On the evening of the same day and on the third and fifth days after operation, the animal was given each time an intravenous injection of from 14 to 20 cc. of fresh 1 per cent. aqueous trypan blue.

The animal was killed later in the afternoon of the fifth day and the tissue preserved in formol. Frozen and paraffin sections showed: (1) Endothelial giant cells lying in the intralobular hepatic capillaries. These were sometimes multinucleated and contained phagocytized albumen flakes. In all cases they stained intensely with the vital stain, and this fact, together with their occasional structural continuity with the neighboring capillary endothelium, demonstrated their endothelial nature; (2) The endothelial lining of those branches of the portal tree which were plugged with emboli showed marked proliferative changes.

This newly formed endothelial tissue existed at the site of the embolus and in its immediate neighborhood and partially occlude the lumen of the vein. It is stained vitally, a phenomenon never seen with the normal endothelial cells of large vessels, and showing that the vital stain is adequate for the detection of endothelial growths although the parent tissue does not show this property.

It is possible in this way to distinguish the rôle of endothelium in contrast to that of the mono-nuclear blood cells certainly in all intravascular lesions.

4. *Demonstration of fat in the muscle fibers of the myocardium and of the atrio-ventricular system.* H. HAYS BULLARD.

The normal fat content of cardiac fibers and of muscle fibers of the atrio-ventricular system was shown by tissue sections which had been treated with fat stains. A stained preparation of the true interstitial granules of myocardial fibers was also shown.



5. *Specimen illustrating the development of the pancreatic duct-system in the pig.* GEORGE W. CORNER, Johns Hopkins Medical School.
6. *Pyridine silver preparations of the vagus nerve of man, dog and cat.* M. R. CHASE, Northwestern University Medical School, Chicago.
7. *Microscopic preparations showing morphological and staining characteristics of the nuclei of lymphatic and blood vascular endothelium and of the mesenchyme cells in chick embryos.* ELIOT R. CLARK, Johns Hopkins Medical School.
8. *Microscopic slides illustrating experimental studies of mesothelium.* WILLIAM COGSWELL CLARKE, Columbia University.
9. *The staining of mitochondria in human lymphocytes with janus green.* E. V. COWDRY, Johns Hopkins Medical School.

Mitochondria were stained, intravital, in the lymphocytes of freshly drawn human blood with a solution of 1 : 10,000 janus green (diethyl safranin-azodi-methyl-anilin) in physiological salt solution.

Mitochondria were also demonstrated in the lymphocytes of blood cells in smears fixed in 2 per cent. osmic acid and Bensley's acetic osmic bichromate mixture, and stained by the regulation Altmann method and by Bensley's anilin fuchsin methyl green method.

The mitochondria seen with the aid of the vital dye and those in the fixed and stained preparations showed a striking similarity with respect to their relative number, shape and cytoplasmic distribution.
10. *Preparations illustrating vital staining with various benzidine dyes.* HERBERT M. EVANS, Johns Hopkins University.
11. *The melanophores of tadpoles.* DAVENPORT HOOKER, Yale University.

The melanophores of both larval and adult frogs were demonstrated in fixed preparations to show their component parts, structure and relation to the spaces which contain them. The larval melanophores showed the even distribution of the pigment in the expanded phase and the absence of processes in the contracted phase. The adult melanophores showed the nature of the processes and their absence in complete contraction of the cell. In both the larval and adult, the spaces within which the cells lie were demonstrable and their relation to the cells, especially in the adult, clearly to be seen.
12. *Teased preparations showing complete seminiferous tubules of Mammalia.* G. CARL HUBER, University of Michigan, Ann Arbor, Mich.
13. *Microscopic slides and charts illustrating the genetic relations of lymphatics and hemal vascular channels in the embryos of amniotes.* GEORGE S. HUNTINGTON, Columbia University.

14. *A demonstration of certain embryonic vessels of amblystoma, necturus and frog.* HENRY MCE. KNOWER, University of Cincinnati.
15. *A syringe for injecting tissues, giving a continuous flow at any desired pressure.* FREDERIC P. LORD, Dartmouth Medical School, Hanover, New Hampshire.
16. *Microscopic slides illustrating experiments on the development of the blood vessels in the blastoderm of the chick.* A. M. MILLER AND JOHN MCWHORTER, Columbia University.
17. *Pyridine silver preparations of the spinal cord of the cat and Rhesus monkey.* S. WALTER RANSON, Northwestern University Medical School, Chicago.

18. *Preparations showing the vital stain applied to the study of wound-healing.* KATHARINE J. SCOTT, Johns Hopkins University.

It has been found that the injection of benzidine dyes results in the deposit of granules in the cells of connective tissue, and that these cells carrying granules are of two types, corresponding in turn to true fibroblasts or fixed cells and to the cells spoken of as "resting wandering cells" or clasmotocytes. In view of this observation, it has seemed important to study the process of wound-healing in animals receiving large amounts of the dye, and to study especially that stage of the process during which new connective tissue is formed, in the hope of throwing new light on the questions of the function and specificity of these cells under given pathological conditions. For this purpose knife wounds have been made in the skin, liver and kidney of rabbits stained with trypan blue. As demonstrated, wounds in the liver of 6 to 7 days duration show extensive necrosis of liver parenchyma in the region immediate to the line of incision. The dead liver cells, nucleus and cytoplasm, stain a diffuse blue, except in the center of the necrotic area, where the dye does not penetrate. The microscopic pictures resembles that of a small abscess, the unstained portion showing strong contrast to the blue-black organ. Microscopically, the dead tissue is surrounded by masses of granule-laden phagocytic cells which appear to have arisen *in situ* from pre-existing endothelial cells, or Kupffer cells, a reaction apparently homologous to that observed in tuberculous livers.<sup>1</sup> These phagocytic cells appear very like the macrophages of the peritoneal cavity. New tissue, arising in adhesions of the mesentery near the wound, shows fibroblasts of embryonic character, with typical fine, blue granules, as well as the cells of polyblastic character with coarse granules. A wound of the same duration, in the kidney, gives a very different picture. The line of incision is filled with new connective tissue, the cells of which are

<sup>1</sup> Evans, Bowman, Winternitz: An experimental study of the histogenesis of the miliary tubercle in vitally stained rabbits, Jour. Exp. Med., 1914.

strikingly free from dye granules, in spite of the abundance of dye-containing cells in the surrounding kidney tissue and capsule. Necrotic cells of injured tubules are present without any of the striking phagocytes seen in the liver wounds.

19. *Microscopic slides illustrating early stages of vasculo-genesis in the cat (Felis domestica).* H. VON W. SCHULTE, Columbia University.

20. *Preparations and drawings showing the results of a modified Weigert technique applicable to serial paraffin sections of the adult human brain.* RALPH EDWARD SHELDON, Department of Anatomy, School of Medicine, University of Pittsburgh. (Full description of methods to be published in *Folia Neuro-Biologica*, vol. 8).

21. G. L. STREETER, University of Michigan.

a. Six sections showing labyrinths in tadpoles that had been transplanted in each case from other specimens at an early stage. In spite of the fact that in the transplantation they were placed intentionally in an inverted position, they developed, as shown by the sections, in the normal manner and in the normal posture. That is to say, they corrected their displacement.

b. A blue print catalogue of about 300 lantern slides. The blue print sheets, showing mostly six slides to the sheet, are mounted in a loose leaf note-book holder.

c. A dry-preparation of the laryngeal cartilages, together with the hyoid bone and the upper four tracheal rings. This mount was prepared in the manner described in the paper given in the general program under the title "Technical experiences".

22. *Microscopic slides illustrating a case of hemocerebellar atrophy.* O. S. STRONG, Columbia University.

23. *Microscopic slides and reconstructions illustrating the morphology and development of the diencephalon in mammals.* FREDERIC TILNEY, Columbia University.

24. *Specimens bearing on the nature of fat cells.* H. G. WEISKOTTEN AND H. S. STEENSLAND, Syracuse University.

25. *Microscopic slides illustrating the development of the caudal lymph hearts in the chick.* RANDOLPH WEST, Princeton and Columbia Universities.

26. *Specimens showing vital staining of the interstitial cells of the testis.* R. H. WHITEHEAD, University of Virginia.

27. *Models illustrating the development of the gall bladder and bile ducts in amblystoma.* E. A. BAUMGARTNER, Department of Anatomy, University of Minnesota.



28. *Reconstructions illustrating the development of the suprapericardial body in Squalus acanthias.* W. E. CAMP, Institute of Anatomy, University of Minnesota; (presented by R. E. Scammon).

The bodies in question were discovered by Leydig in 1853 in *Anuria*, and were described as accessory thyroids. Van Bemmelen (Mitt. Zool. stat. Neapel, Bd. 16, '86) first described these structures in elasmobranchs as 'suprapericardial bodies.' He considered them to represent rudimentary seventh pouches.

The models were made in two series: Series A, to show the position of the bodies, and their relation to the pharynx and its derivatives; Series B, at a much higher magnification to show the form and later development of the bodies.

Series A, Model 1. Reconstruction of the pharynx of a 20.6 mm. embryo (H.E.C.1494).<sup>1</sup> The suprapericardial body is present only on the left side where it is represented by a simple thickening of the epithelium of the ventral pharynx wall. It extends through four sections of 10  $\mu$  and lies at the same level as connection of the sixth pouch of the left side with the pharynx.

Series A, Model 2. Reconstruction of pharynx of 33.1 mm. embryo (S.C.S). The suprapericardial bodies are present on both sides. The body on the left side is very much the larger. The right body in this embryo is represented by two slender villus-like projections of the epithelium extending down into the mesenchyma between the pharynx wall and the pericardium. They have no lumina and are found only in three sections of 12  $\mu$ . The body on the left side consists of a single, fairly well developed cyst which is connected at right angles with a stalk which has a lumen continuous with the cyst and is connected with the pharyngeal epithelium. The bodies are in about the same relative position as in the preceding embryo, that is, a little posterior to the beginning of the ventral diverticulae of the sixth pouch and about halfway between this pouch and the median line.

Series A, Model 3. Reconstruction of the posterior part of the pharynx of a 47.3 mm. embryo (S.C.11.) showing the fourth, fifth, and sixth pouches. In this stage, due to the growth of the pharynx, the bodies have a more lateral position. As in the preceding embryo, the body on the left side is the more highly developed. The form of the bodies at this stage is described in Series B.

Series A, Model 4. Reconstruction of posterior half of the pharynx of 95 mm. embryo of *Squalus sucklii* (H.E.C.1882). The position of the bodies at this stage is a little more median than in the one preceding. The bodies, particularly the one on the left side, are turned toward the midline from their origin from the pharynx wall. Arising just lateral to the posterior extremity of the body on either side of the pharynx and running downward, outward and forward, is an elevated crest which ends on the right side in a fold of the esophagus and on the left side termi-

<sup>1</sup> H.E.C.=Howard Embryological Collection; S.C.=Embryological Collection of Dr. R. E. Scammon.

nates just anterior the junction of the flattened and expanded pharynx with the esophagus.

Series B, Model 1. Reconstruction of the left suprapericardial body of a 28 mm. embryo (H.E.C.1357). The body consists of a single cyst connected with the pharynx wall by a hollow stalk. The cyst is directed anteriorly.

Series B, Model 2. Reconstruction of left suprapericardial body of 37 mm. embryo (H.E.C.363). The body is composed of a slender cyst, pointed at its anterior extremity, and constricted at its middle. It is connected to the pharynx by a budding and contorted hollow stalk, which opens into the base of the cyst.

Series B, Model 3. Reconstruction of right suprapericardial body of 47.3 mm. embryo (S.C.11). This body is of about the same stage of development as the left body of the 28 mm. embryo.

Series B, Model 4. Reconstruction of left suprapericardial body of 47.3 mm. embryo (S.C.11). The body at this stage consists clearly of two parts. The first a single elongated cyst extending antero-posteriorly and widely connected with the pharynx. This is undoubtedly the remnants of the early connecting stalk. The second part, larger and separate from the first, consists of a branching and budding mass of tubules, the lumina of which appear to be continuous.

Series B, Model 5. Reconstruction of the left suprapericardial body of 95 mm. *Squalus sucklii* embryo (H.E.C.1882). The body in this embryo consists of a complicated mass of branching tubules some of which anastomose. They are placed at an angle of about 45° to the pharynx wall. Remnants of the stalk are seen in three distinct solid cords connected with both the mass of tubules and with the pharynx wall. The greater part of the body extends anterior to its attachment to the pharynx. A few small isolated cysts are present lying in close contact but apparently not connected or fused with one another. The ventral boundary of the body is sharply defined by a large elongated cyst which is pointed at its anterior and bifurcated at its posterior extremity.

*Summary.* (1). The suprapericardial bodies develop from the ventral pharynx wall medial and at about the level of the sixth pouch. (2) The left suprapericardial body develops progressively with the pharynx and its appendages. The right suprapericardial body is not present in all embryos and when present is always smaller and more rudimentary than the left one. (3) In the later stages the bodies consist of branching tubules some of which anastomose, and of a few isolated cysts. (4) The later growth of the bodies is directed anteriorly and is independent of the pharynx. (5) None of the bodies in the embryos examined show any fusion with the thyroid.

*29. Eosinophilic leucocytes and hematogenous mast cells in the bone marrow of the guinea-pig.* HAL DOWNEY, University of Minnesota.

The demonstrations consisted of slides and drawings which illustrate the heteroplastic development of the various types of granular leucocytes in the bone marrow of the guinea-pig from non-granular cells. Besides

this the slides show that there are many polymorphonuclear mast cells and the corresponding myelocytes in the marrow of guinea-pig, which is contrary to the findings of Benacchio and Kardos, who state that there are no mast leucocytes in this marrow and no intermediate stages between mononuclear cells with coarse basophilic granules and polymorphonuclear mast cells.

The preparations were smears of bone marrow which were fixed in Helly's Zenker-formol mixture while they were still moist. They were stained in the May-Grünwald mixture, in Ehrlich's triacid and indulin-aurantia-indulin mixtures, and in Dominici's combination of eosin-orange G-toluidin blue. With all of these methods the mast leucocytes and their myelocytes were easily distinguished from the eosinophil leucocytes in all stages of their evolution.

30. (a) *Models of the gastric mucosa in rat, pig, and sheep embryos; (b) Electrically heated instruments for making wax models; knife for cutting plates smoothing-iron.* CHESTER H. HEUSER, The Wistar Institute of Anatomy.
31. *Model of a 2 mm. human embryo.* N. W. INGALLS, Western Reserve University, Cleveland, O.
32. *Plans for a new anatomical laboratory in Cincinnati.* HENRY McE. KNOWER, University of Cincinnati.
33. (a) *Models showing the escape of ovum from uterine cavity in Geomys; (b) An improved microscopic lamp.* THOMAS G. LEE, University of Minnesota.
34. *Degeneration in thirty different segments of the spinal cord of a dog, following a mesial section for one centimeter at the level of the cervical nerve.* BURTON D. MYERS, Indiana University.
35. (a) *Model of the conus of the frog's heart; (b) A trilocular heart with pulmonary stenosis; (c) Sections through the canalis craniopharyngeus of the rabbit; (d) Sections of auricular region of newborn infant.* A. G. POHLMAN, St. Louis University.
36. *Models illustrating the development of the pancreas in Selachians.* R. E. SCAMMON, University of Minnesota.





# THE OCCURRENCE OF SUPERNUMERARY SPLEENS IN DOGS AND CATS, WITH OBSERVATIONS ON CORPORA LIBERA ABDOMINALIS

## IV. STUDIES ON HEMAL NODES

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### TWELVE FIGURES

In a report on 80 autopsies on infants published in 1895, Jolly (12) states that he found supernumerary spleens present in 25 per cent of the cases. In 2 of the 8 cases two supernumerary spleens were found. The supernumerary spleens in these 8 cases were irregular in form, generally polyhedral and of the size of a hazel nut. In the other 14 cases they were regularly rounded and easily distinguished from lymph nodes by their color which was that of the main spleen. They were usually located on the inferior surface of the gastro-splenic ligament.

If Jolly's findings in these 80 autopsies on infants and children, ranging in age from the newborn to fourteen years, can be considered as representative, then the experience of most anatomists would, I think, confirm the old statement that supernumerary spleens in man are much more common in early than in late life. Haberer (8) also came to this conclusion which would seem to be contradicted by Jolly's statement that there is an apparent augmentation in volume with increasing age and that if such is the case accessory spleens are apparently not transitory organs. No doubt Grëveilhier's conclusion that for various reasons they are more difficult to recognize in the adult is correct; but it seems open to question, whether an actual degeneration or even an atrophy due to age, takes place as Picou (15) thinks is indicated by the fact that it is the rule for accessory organs to atrophy and disappear completely with age. The only direct evidence suggesting a possible atrophy of supernumerary spleens obtained in

the examination of the series of domestic animals reported on here, was found in the case of certain peculiar and questionable pedunculated masses described below. However, in these appendices the abnormal circulatory conditions arising from the presence of a long, slender, occasionally twisted pedicle might well be responsible for the fibrosis which existed.

The lack of information regarding the occurrence of accessory spleens in some of the domestic animals—especially dogs and cats—became fully apparent to me some years since in examining the literature on the experimental production of spleens and so-called hemolymph nodes. Moreover, since none of the investigators who reported the experimental production of hemolymph nodes or spleens, as judged by their own publications, had adequately informed themselves upon this very important question, the conclusions which they reached regarding the new formation of spleens in the omentum or on the peritoneum, after splenectomy, are necessarily open to serious doubt. Foa (4) was the first to question the validity of Tizzoni's (18, 19) conclusions regarding the new formation of accessory spleens after splenectomy. Tizzoni, it will be recalled, excised the spleen in four dogs and found 60 to 80 accessory spleens in the great and gastro-hepatic omenta 2, 54 and 90 days respectively, after operation. Although no examination of these animals had been made before operation, Tizzoni for wholly insufficient and incorrect reasons nevertheless concluded that these spleens had formed *de novo*. Foa, who had also called attention to the fact that such small nodules as described by Tizzoni are also found in dogs with entirely *normal* spleens, apparently did not publish anything further on the subject, but Tizzoni, who was very evidently piqued, was roused to great activity, for he published the results of 40 necropsies a few weeks after the formal presentation of Foa's suggestions. In this paper Tizzoni (20) lays special emphasis on the fact that these necropsies were done at the Veterinary School of Bologna in the presence and with the aid of his excellent friend Gotti.<sup>1</sup> The astonishing thing is that although 262 accessory

<sup>1</sup> The inference is clear, of course, and I mention these details simply because Tizzoni's experiments have been accepted for decades.



spleens were found in four out of these forty dogs Tizzoni nevertheless says that all his previous conclusions are confirmed! Tizzoni believed that in these four dogs nature herself had done a partial splenectomy by the development of chronic interstitial splenitis. Moreover, he performed splenectomy on a dog that had accessory spleens and although this animal died of an infection "before the usual results could manifest themselves" he nevertheless concluded that the results obtained in this animal were sufficient wholly to warrant his former conclusions! Tizzoni also failed to state what the results in this case were and then stated that the accessory spleens which form as a result of splenectomy always arise as Malpighian corpuscles which become surrounded by pulp later, while on the contrary, in the case of those that arise as a result of pathological conditions, the pulp forms first and the rest of the spleen from it.

In May, 1882, Griffini (6) reported a series of experiments on the regeneration of small, experimentally-produced defects of the spleen in 14 dogs and added that in a few cases he noticed a new formation of spleens from the great omentum or from the spleen itself *because of conditions not yet determined*. However, Griffini also failed to give any further details regarding these accessory spleens.

A year later Tizzoni (21) reported that he did splenectomies on two dogs which possessed accessory spleens before operation "as a result of disease." In one animal which was killed six months after operation there was no increase in size and number of the accessory spleens previously present in the great and gastro-splenic omentum, but there were many newly formed spleens in the gastro-hepatic omentum, in the lateral ligament of the bladder, in the plica Douglasii, in the ischio-rectal fossa, in the serosa of the stomach and in the central tendon of the diaphragm. In the second animal killed seven months after splenectomy, Tizzoni concluded that there was *perhaps* an increase in size and number of the accessory spleens previously present in the great and gastro-splenic omentum and that in addition other newly formed spleens in all stages of development were found as in the previous animal. Since the first animal had suffered from post-

operative local peritonitis Tizzoni concluded that these inflammatory processes inhibited the formation of accessory spleens in the gastro-splenic and great omentum and lead to their formation elsewhere.

Even granting that Tizzoni was still unbiased and not on the defensive, it is perfectly evident, to be sure, that all the additional accessory spleens which Tizzoni regarded as having formed *de novo* because of splenectomy may just as well have formed because of conditions responsible for the existence of accessory spleens before the operation. Hence splenectomy may not have been a factor at all. The same objection holds for the increase in size.

A year later Tizzoni (22) published a series of conclusions regarding accessory spleens drawn from 60 autopsies, details regarding which he does not give. These conclusions repeat all the older conceptions regarding accessory spleens and emphasize the fact that the new formation of accessory spleens always results from a chronic interstitial splenitis. Tizzoni also draws further distinctions between spleens formed experimentally and as a result of pathological conditions. Since no new facts are presented in this two-page article and since many of Tizzoni's conclusions can easily be shown to be untenable they will not be discussed here.

During June, 1883, and in 1884 Griffini and Tizzoni (7) reported that 'in some instances,' they found newly formed nodules on the main spleen and in the great omentum in a series of 97 partial excisions of the spleen. The pieces excised were small (4-15 by 5-20 mm.) and the dogs were killed from 40 hours to 89 days after operation. In this article which the authors call a *résumé*, they give no details whatever, nor do they rule out reddened lymph glands. This joint *résumé* was followed by another *résumé* by Tizzoni (23) in which he declared that he wanted to test especially what would happen if splenectomy were done on dogs in which accessory spleens had previously formed as a result of disease of the main spleen. This he considered necessary in spite of the fact that Tizzoni had on at least two previous occasions experimentally tested and reported definite—very definite—conclusions

regarding this very matter. Tizzoni again gives no details regarding these dogs—how they were selected, how many and how large the accessory spleens, how they were measured and where located, etc., etc., yet he reported that he found an increase in the number and volume of the nodules previously present in the gastro-splenic and great omentum of the two dogs on which splenectomy was done. The experiment was limited to two dogs—“*Ver la difficulté d'avoir des chiens qui se trouvent dans les conditions requises.*” The same objection urged above applies, to be sure, to these two experiments for in these cases also the conditions responsible for the formation of accessory spleens before operation may have been responsible for their continued formation after splenectomy unless one assumes what Tizzoni does not that the effect of these processes is limited entirely to the main spleen, for Tizzoni reported the existence of identical pathological processes in accessory spleens. Hence it seemed likely that Foa was correct when he suggested what Luschka had previously asserted, that accessory spleens are found in animals having wholly normal spleens.

In a final article Tizzoni (24) reported on 29 dogs in only four of which he found accessory spleens and never more than three in any one animal. The interesting thing in this article is the finding of the same pathological processes in the accessory as in the main spleen and the existence of a macroscopic zone of infiltration around some of the accessory spleens. Tizzoni again concluded that these findings confirm all his previous conclusions.<sup>2</sup>

To determine the incidence of accessory spleens a large series of cats and dogs were carefully examined in connection with studies on hemolymph nodes in the domestic animals. The results were quite surprising for in a series of 98 cats of both sexes, of all ages, in all stages of pregnancy and in varying states of health and nutrition six contained one supernumerary spleen, one contained two, and one each 6, 59, 160 and 195 supernumerary spleens respectively. Hence 11.2 per cent of these cats contained accessory spleens and the total number of spleens found in these

<sup>2</sup> A fuller discussion of the experimental production of accessory spleens and hemal nodes by the writer will be found in the *Jour. Exp. Zool.*, February, 1914.



thirteen animals was 425. The ages of the cats in which the accessory spleens were found varied approximately from two to seven years and was considerably higher than that of the dogs. None were found in very young cats although a careful search was made, and although the total number of animals examined to date is 98, no relation between the occurrence of supernumerary spleens and sex, pregnancy or any other condition was noticed.

The overwhelming majority of the accessory spleens were located on the great omentum but in one case in which six were present all were found near the hilus of the main spleen, on the dorsal surface of the gastro-splenic omentum. Most of them were very small—0.5 to 2 mm.—and many of microscopic size only. A few of the larger ones had the gross appearance of spleens but the rest all looked exactly like hemal nodes. The large ones usually lay near the main spleen or a small portion of the main spleen which was more or less distinctly marked off from the main mass as a separate lobule, and it is evident that such occasional findings as these would naturally suggest the old conception of fragmentation and daughter spleens.

In the cats in which such a large number of accessory spleens were found a number were often grouped closely together or were fused, so as to form small aggregates but the individual nodes were seldom more than 1.5 to 2.5 mm. in size. The largest were oval but the smallest were spherical specks, many of which could only be distinguished clearly with a hand lens.

In the three cases in which so many supernumerary spleens were present both surfaces of the great omentum were completely studded with them and in one case a few of the larger isolated specimens or groups, which lay in the immediate vicinity of, or even adjacent to the main spleen, might properly be designated as daughter spleens were it not for the questionable relationship thus implied. In two of the three cats having such large numbers of accessory spleens omental adhesions were present on the main spleen and the latter was scarred in one case, but in the third cat the spleen was wholly smooth and normal. In the first two which were household pets the main spleens were soft, large and deeply red but in the third cat it was small, firmer and

paler. The disproportion in size between the first two and the third was marked even after allowing for the differences in size of the cats. One of the large spleens also had a distinct lobule about 2.5 cm. square which was attached by its base, but which had no separate blood supply and in no sense formed a transition between the main and the accessory spleens. None of the latter were surrounded by a hemorrhagic area and there was no reddening of the lymph nodes although one or several lymph nodes which could not be distinguished from accessory spleens because of their red color, were found. By means of injections these were shown to be lymph nodes.

In five cats small pedunculated nodes which were first labeled hemorrhagic tags of the omentum were also found, but these because of their bizarre form and somewhat different structure, should perhaps not all be included among supernumerary spleens. The pedunculated masses recalled the two pedunculated supernumerary spleens mentioned by Albrecht (1) as having been found upon the peritoneal surface of the upper portion of the rectum in man and "the very small round nodule" found by Schilling (17) on an appendix epiploica of the descending colon as well as the "almost pedunculated nodules" mentioned by Tizzoni (19). The two pedunculated nodules mentioned by Albrecht were said to have the size of a hemp seed.

In several instances the pedicles of the small occasionally pyriform nodules found in these cats were relatively long and so slender as to be barely visible to the unaided eye. The gross appearance of these hemal omental appendages in both the dog and cat, is well shown in figures 1 and 2. Although the short, thick fatty pedicle of the one from the dog shown in figure 2 is directly continuous with the fat of the great omentum, it and its nodular hemorrhagic extremity are free and rest on a very thin and fenestrated portion of the omentum. On the dorsum of this pedicle a vein which ended in the hemal nodule was easily seen with the unaided eye but no artery was visible anywhere. The demarcation between the nodule and the fat of the pedicle was very sharp in this instance but such is not always the case, as is well shown in figure 1, which represents a much smaller but similar

nodule from the cat. The pedicle of this appendage was both relatively and absolutely longer. It was also of very much smaller caliber but likewise contained a small vein which although somewhat spirally arranged because of the twisting of the pedicle, was nevertheless distinctly visible in part of its course as is shown in the illustration. The actual measurements of the node after fixation were 1 by 1.5 mm. No line of abrupt demarcation between the nodule and the pedicle which is 8.5 mm. long is evident and there is also a gradual transition in color and a change in form

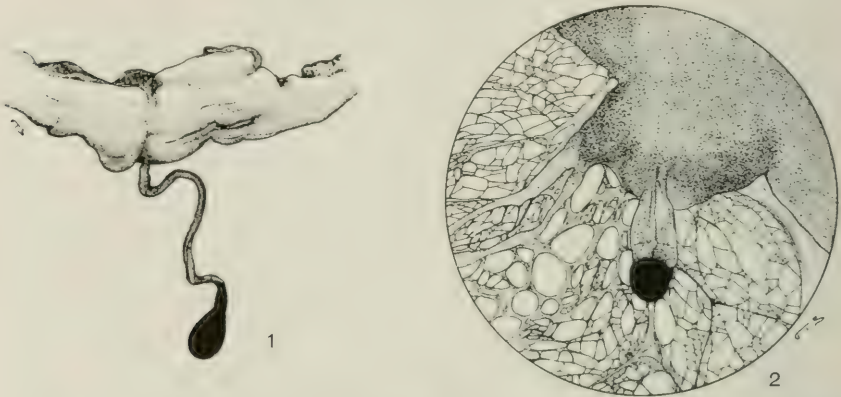


Fig. 1 A hemal omental appendage from the cat, showing a portion of the spirally arranged vein and of the omental fat to which the fatty pedicle is attached.

Fig. 2 Hemal omental appendage from the dog. The short pedicle arising from the omental fat lies on fenestrated omentum. Note the vein on the dorsum-

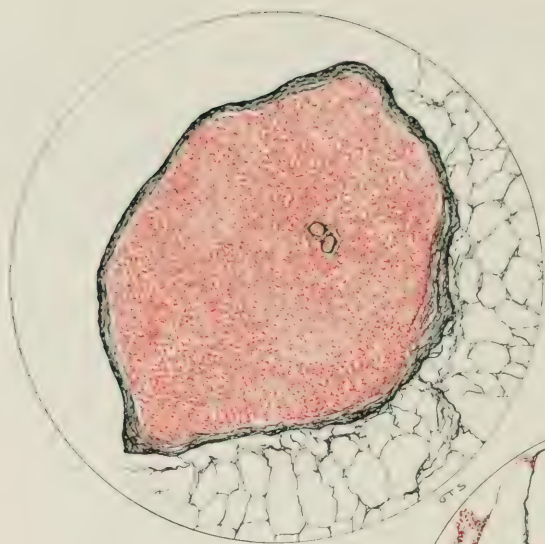
from the one to the other. However, these characteristics are not at all unusual.

These small and peculiar appendages were found in various locations on the external surface of the omentum but never on the internal surface in the bursa omentalis. However, since no very extensive series of animals—approximately one hundred—has been examined such a location cannot be excluded with certainty.

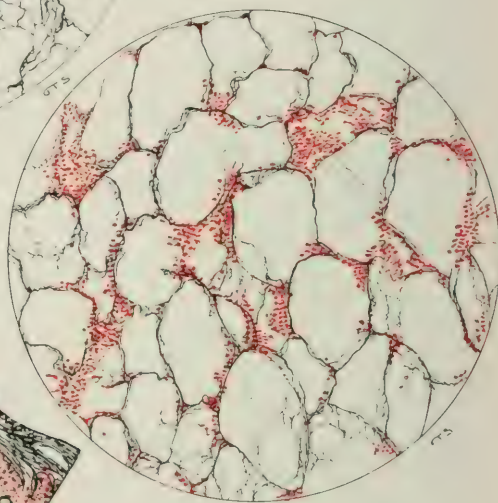
The distribution as noticed in these specimens may not be without significance in the origin of some of them. That all of



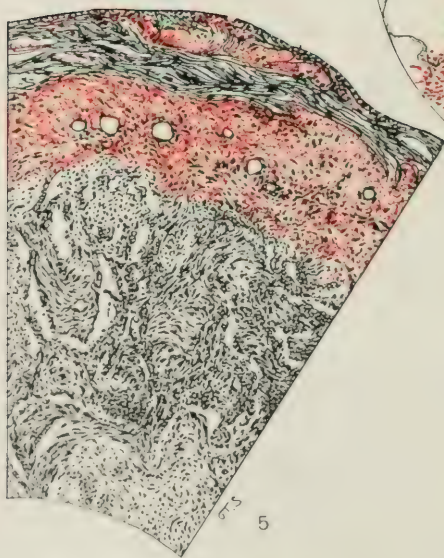
them are in very intimate relationship with the surrounding fat is evident from gross examination alone and in serial sections it is seen that in some of them there is no continuous or definite fibrous capsule to delimit the fat from the node. In others, however, there is a very definite connective tissue capsule as can be seen in figure 3 which represents a cross section of the more central portion of the node from the dog, shown in figure 2. In this node a very definite fibrous capsule which was covered by peritoneum on its free surface and bounded by the underlying fat on the other, encloses a mass of blood in which two small somewhat eccentrically placed vessels are found. The whole vascular sac is devoid of trabeculae at this point and not a leucocyte or lymphocyte can be found anywhere among the large mass of erythrocytes. The blood which is well-preserved contains no pigment and there are no evidences of destructive changes. In the more proximal portions a more or less well-defined capsule is also present but still further proximally the adipose tissue is completely infiltrated with blood and finally the infiltration becomes much slighter and most of the erythrocytes lie near the intercellular boundaries as shown in figure 4 which represents a cross-section of the pedicle in a similar appendage from a cat. Here the infiltration with erythrocytes is comparatively slight but it becomes still slighter as one proceeds still further proximally until there are no evidences whatever of infiltration and all the blood is contained in vessels. Hence in some of these cases there is a gradual transition from the surrounding fat to conditions as represented in figures 3 and 4 and finally more distally to a structure as represented in figure 5 which shows a portion of a transverse section of the same appendage directly through the node. The striking thing about this section is the presence of an exceedingly large amount of connective tissue especially in the center of the node and of numerous small vessels near the periphery in the vascular area. However, there is very little connective tissue in this area which is bounded externally by a diffuse capsule of considerable thickness with much blood between the irregular layers of the connective tissue which form it.



3



4



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Fig. 3 Cross section of an omental appendage from a dog. Same as figure 2. Practically a total absence of leucocytes.

Fig. 4 Cross section of the more proximal portion of the pedicle of a similar appendage from a cat. Note the distribution of the blood.

Fig. 5 Cross section directly through the node a cross section of the pedicle of which is shown in figure 4.

From the accompanying illustrations it is evident that some of these pedunculated hemal omental appendages which are really appendices epiploicae in the true sense of the word, resemble supernumerary spleens only more or less remotely structurally, and probably are not such. Since no satisfactory series of transition forms were obtained their genesis must remain largely a matter of surmise. However, the hemorrhagic nature of the adjacent fat not infrequently seen, and the transition from this to entirely normal adipose tissue naturally directed special attention to small ill-defined hemorrhagic areas occasionally found in various portions of the omentum. These small areas are usually somewhat elevated and nodular and it does not seem unlikely that further congestion whatever its cause, accompanied by or resulting in diapedesis or hemorrhage and fibrosis might account for some of the non-pedunculated hemal nodes. The fibrosis could, it seems, be attributed to congestion, infiltration and disintegration of blood cells although no such disintegration was observed. The erythrocytes were well-preserved. However, since several undoubted specimens of pedunculated supernumerary spleens were met with in animals possessing large numbers of these organs and especially since some of the larger isolated supernumerary spleens which also resembled the main spleen in color and surface appearance had a very short pedicle the possibility that some of the hemal nodules on these omental appendages are really spleens which have undergone fibrosis must be borne in mind.

An inflammatory origin did not seem at all probable, but whatever the genesis of these hemal omental appendages it is evident that only slight tension or torsion would rupture such slender pedicles as those represented in figure 1 and thus convert the nodule into a free body or so-called corpus liberum. Although it is not probable that such extremely small vascular nodules as these would give rise to any disturbances because they would undoubtedly be absorbed rapidly, yet in case of the much larger appendages represented in figures 6 and 7 such would probably be the case. These two constricted fatty appendages were observed on the omentum of a dog and throw considerable light





6



7

Fig. 6 Fatty omental appendages from a dog. Natural size.

Fig. 7 Longitudinal section of the fatty omental appendages shown in figure 6. The circular portion of the smaller one shows the partly calcified area. Natural size.

on the genesis and origin of corpora libera adiposa in the abdominal cavity.

According to Virchow (26) free fatty bodies in the abdominal cavity arise as a result of elongation and torsion of the pedicles of appendices epiplocae—appendices coli—in consequence of the traction from peristalsis. Before the appendices which have been converted into polyp-like structures, become detached, atrophy of the appendix and a thickening of its capsule is said to take place. As a result of the latter the capsule is said to become stratified and even cartilaginous in nature. In consequence of obstruction to and atrophy of the bloodvessels which supply the appendices, a fatty degeneration with subsequent cyst formation and possibly calcification is also said to occur.

Riedel (16) called attention to the fact that a demarcation occurs at the place of torsion, that the distal portion of the appendix is likely to be affected by inflammatory processes and that it may hence become adherent and form bands which may give rise to ileus. Riedel supports this opinion by several clinical cases and it is particularly interesting that the band in one of these cases in which a free body was present in the abdominal cavity, was 'reddish' in appearance. Microscopic examination, strangely enough, is said to have shown it to be composed of nothing but fat. That the assumption of Riedel regarding the formation of bands by the torn pedicles is probably well-founded is indicated by the writer's observations to the effect that the distal free extremity of the degenerating umbilical vein in the dog and sheep not infrequently becomes adherent to the parietes or to some viscus, and thus forms a band which may persist for considerable periods of time. The detached omphalomesenteric vessels may likewise obtain a secondary attachment<sup>3</sup> before finally disappearing and the same holds for the fringe on the irregular margin of the wide and prominent projecting fold of extra-peritoneal fat lying in the median line and extending from the xiphoid to near the umbilicus in the cat, dog, etc. But the surprising thing in

<sup>3</sup> Vide Meyer, Retrogressive changes in foetal vessels and ligaments. *Am. Jour. Anat.*, 1914. Some observations and considerations on the umbilical structures of the newborn. *Am. Jour. Obstetrics*, 1914.

connection with these bands so frequently seen in dogs and cats. is not their presence but the fact that they *apparently* cause little trouble, a fact which may, to be sure, be dependent upon the horizontal attitude of the animals.

Since the cases of corpora aliena adiposa abdominalis found in the literature as far back as 1875, have been recently reviewed by Müller (14) no further comment will be made on the clinical aspect of this subject. From Miller's review and from a splendid discussion and review by Hoche (11) it is clear that heretofore practically all cases were regarded as arising from detached appendices epiploicae (appendices coli). An exception, however, is the case of Elter (3) in which a large number of smaller and larger calcified nodules, some of which were almost the size of a walnut, were found in the great omentum and the mesentery. Besides, a similar oval nodule  $4 \times 3 \times 2$  cm. and weighing 21 grams had, previous to necropsy, been removed operatively from the rectum where it was thought to have gained a secondary attachment. Elter concluded that the latter had become separated from the omentum or mesentery because of elongation and rupture of its pedicle which he thought was formed as a result of gravity. Elter further concluded that these nodules primarily form as fibromata or fibro-lipomata which later undergo necrosis and calcification.

The omental appendages in dogs and cats to which attention has been directed can, however, undoubtedly also give rise to free bodies. This is especially well illustrated by the comparatively

Figs. 8 to 12 Supernumerary spleens from the dog (*Canis familiaris*).

Fig. 8 Smallest (youngest?) supernumerary spleen found in the omentum of a dog. It is in connection with a vein only and contains a few lymphocytes but no free erythrocytes.  $\times 1050$ .

Fig. 9 A portion of a small supernumerary spleen from the omentum of a dog showing the relations of the artery and the variations in the capsule.  $\times 1000$ .

Fig. 10 A section of the smallest supernumerary spleen found containing both a vein and an artery and free erythrocytes.  $\times 920$ .

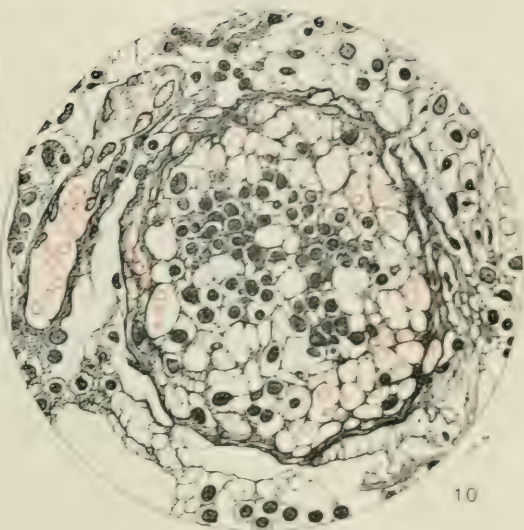
Fig. 11 A portion of a supernumerary spleen showing the absence of a distinct capsule.  $\times 410$ .

Fig. 12 A portion of a supernumerary spleen which is practically a sac of blood. The infiltration of the extra-capsular region with erythrocytes and the relation of the surrounding fat are well shown.  $\times 300$ .

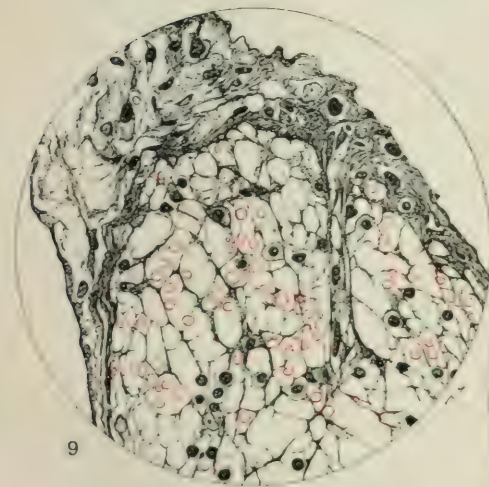




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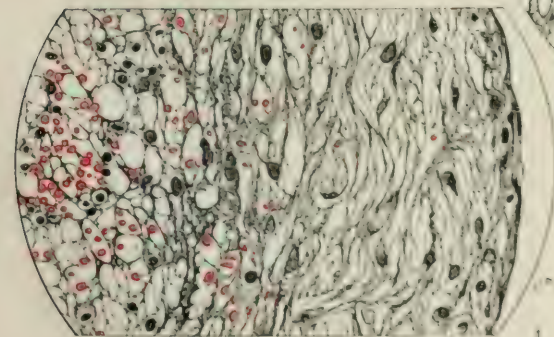
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12



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large, constricted fatty omental appendages from the dog represented in figures 6 and in section in figure 7. The form of both of these seems to suggest that they were moulded somewhat by peristaltic action. That represented in figure 7, which is 6.7 cm. long and approximately 1 cm. in diameter at the base, is slightly flattened and tapers to a somewhat nodular pyriform extremity a little darker in color than the rest. The markedly constricted neck which is only 2 mm. broad and 1 mm. thick is composed only of peritoneum and fat. No vessels whatever are visible in it or in the appendix itself. Nevertheless, the fat is practically normal in gross appearance and consistency and is not surrounded by a thickened capsule. On the other hand the smaller one represented in figure 7 is much firmer, yellowish in color, contains calcified areas<sup>4</sup> and is enclosed in a somewhat thickened capsule which is cartilaginous in consistency on one side near the base. The largest *apparently chondrified* area is found directly distal to the thin, broad, flattened neck which again is non-vascular and composed almost entirely of serosa.

Since both these appendages are attached by such slender necks it is easy to see that they would ere long have been detached completely and become free bodies. Because of their size it is more than likely that degenerative processes would have given rise to some abdominal disturbances unless calcification could have prevented putrefactive changes. Moreover it seems to me that these specimens show that necrosis and calcification do not necessarily occur before separation of an appendix and that there may be no congestion. They also seem to indicate that fibrosis is not necessarily present and that the pedicles may be so extremely short as to make the formation of bands after separation very unlikely. From several instances in which thickened calcified areas one or more centimeters in size were found present in the omentum it is also suggested that local calcification may be a cause in the formation of some of these appendages and hence may *precede* rather than *follow* appendix and pedicle formation and segregation.

<sup>4</sup> These are contained in the basal circular portion and look a trifle darker in the right half of the smaller appendage in fig. 8.



In a series of 67 dogs of varying ages and conditions two dogs contained 1 and one dog each, 4, 11, 18 and 787 supernumerary spleens respectively. That is, 8.9 per cent of these dogs contained supernumerary spleens and the total number found was 821. The ages of the dogs in which supernumerary spleens were found ranged from four to five months to four to seven years but most of them were young animals, which was not the case in cats. In three of the dogs the supernumerary spleens were limited to the great omentum and in two animals they were practically all surrounded by a somewhat irregular hemorrhagic zone in the middle of which the well-defined nodule lay. In one instance in which only four supernumerary spleens were present all were located in the mesentery but in the dog possessing 787 they were found mainly in the great omentum. Some were, however, located on the gastro-splenic omentum and a number also in a fatless fold of peritoneum extending from the spleen to the diaphragm and on the latter itself. Several were also found in the fat near the left kidney. In two instances pedunculated spleens were present. The size of the supernumerary spleens in the dog varied similarly as in the cat and only a very few of them possessed the characteristic color and surface of spleens. In fact, although less variable in color most of them looked like small hemal nodes. Only a few were more than 1 to 2 mm. in size, and as was the case in cats, all, except four, which were located in the mesentery, were found in the greater or lesser omentum.

Several investigators also reported the finding of supernumerary spleens in the pancreas. In four of these 98 cats small dark red slightly elevated areas were noticed in this organ. Two of these cats were only a few days old and the other two were kittens. In all cases these hemal areas which were only a few millimeters in size were indistinguishable from hemal nodes or supernumerary spleens macroscopically. Upon microscopical examination they were, however, found to have no capsule and were hence not delimited from the surrounding glandular tissue. In two instances they were composed almost exclusively of erythrocytes which lay in an inconspicuous connective tissue frame



work containing some lymphocytes. All of these areas were irregular and extended between the lobules of the pancreas in various directions. In two cases a small circumscribed area composed wholly of lymphocytes was also contained in them. These had the appearance of lymph follicles and since the surrounding blood contained more lymphocytes than in the other cases this gave to these hemorrhagic areas more of the appearance of splenic tissue. Although no remnants of pancreatic cells were contained in the latter I am nevertheless inclined to regard some of them as due to hemorrhage rather than as accessory spleens. It is true that they are rather atypical, for signs of degeneration of erythrocytes were not present and they contained few leucocytes. The lymph follicles or nodules contained in one are difficult to account for on the basis of hemorrhage but the entire absence of a capsule and the fact that these areas differed from supernumerary spleens in certain other respects make me hesitate to consider them as such for such an interpretation would seem to imply that they arose *de novo*. However, in one cat, an adult female two years old, No. 102, three spleens were found in the right extremity of the pancreas. All were unmistakably spleens. The largest and most prominent was imbedded quite completely, while the two smaller were visible only over an area of 1.5 to 2 mm. of their surface. Their size ranged from 1.5 to 3.5 mm. In the largest about a dozen Malpighian corpuscles were plainly visible on section after fixation. Most of these were of the same size as those in the main spleen which although not sclerotic or pale was unusually small for the size and condition of the animal. There was nothing peculiar about the lymph nodes in this cat or of those in the three kittens to which she had given birth about six weeks previously. All the viscera including the spleen and pancreas were of normal shape and there was no evidence of any developmental anomaly.

Tizzoni spoke of a hemorrhagic zone around some of the accessory spleens found by him in the great omentum of the dog and the same condition was observed by Albrecht in man. In the case of the dog containing 787 supernumerary spleens the

areas of infiltration were very striking indeed. These hemorrhagic zones were so large and irregular in this dog that it was at first thought that they were due to the fact that the animal had been killed by an intravenous injection of chloroform while under chloroform anesthesia. However, in view of the above observations of Tizzoni and Albrecht and moreover since similar hemorrhagic areas were found around nodes in several instances in other animals it does not seem likely that they were due to the intravenous injection of chloroform or to the preceding chloroform anesthesia, although these may have made them more prominent. Upon microscopic examination the hemorrhagic areas show nothing but an infiltration of the surrounding tissue with blood cells. Similar isolated areas are sometimes seen in omenta containing no supernumerary spleens and near hemal omental appendages or in the pedicles of the latter. No gradation from these areas into the hemal nodes was noticed and the latter were almost always enclosed more or less complete by a definite capsule. These infiltrated areas also differed from ordinary hemorrhagic areas in the absence of marked degeneration of erythrocytes, of pigment and of much phagocytosis in certain portions.

The lymph nodes in these animals were normal in size and appearance but, as in the cats, occasionally a very red node was seen. The latter and all the thoracic nodes which were very red in one dog, were found upon injection to be lymph nodes. In some cases these were very pale gray instead of red and nothing was seen differing in the least from what was found in dogs having no accessory spleens. The main spleens were normal and no scars or adhesions or separate lobules were found.

The existence of such large numbers of supernumerary spleens in these apparently normal dogs and cats recalls the pathological case of Albrecht (1) and also the statement of Laguesse (13) that the spleen of several kinds of selachians is normally divided into an infinite number of completely separated red co-adapted perls which project from the mesogastrium. Laguesse also stated that in *Carcharias glaucus* as many as 2000 such nodules have been

counted. However, in this species there is no main spleen in addition and hence it is evident that the conditions are not all comparable to the above conditions in the cat and dog.

In these dogs and cats the main spleens which were quite regular in form save in two cases, varied somewhat in size but in no case were they abnormally small or diseased. Moreover a 'Zer-sprenzung' or disruption of the spleen was suggested remotely in but two cases by the accumulation of some of the small nodules in the immediate neighborhood of the body of the main spleen. In one case a small splenic lobule was also present, and another spleen contained a deep scar but in several cases in which very definite and comparatively large cicatrices unaccompanied by adhesions (pseudo cicatrices?) were present no accessory spleens were found. Nor were there any displacements or deformities present in any other organ as in the case of the human being reported by Albrecht. In the latter case in which 400 spleens were scattered all over the peritoneum Albrecht referred their origin to the occurrence of some mechanical disturbance during development. No corroborative evidence for such a supposition was obtained in this series of animals, however, nor was anything seen confirming Schilling's (17) supposition that defects in the spleen are usually associated with anomalies of the circulatory system.

From observations on the spleens of a considerable series of domestic animals it became apparent that some of the so-called scars are undoubtedly pseudo-scars. It was noticed for example that the surface of some spleens showed slight local pitting or ridging of varying degrees. In some cases these fibrous depressions or elevations were irregular with smaller sulci and folds extending laterally from the main sulcus. Such purely capsular folds and depressions can simulate scars very closely, indeed, and hence easily lead to misinterpretations. At any rate, I am firmly convinced that not everything that even closely simulates a scar, is actually such, i.e., has a traumatic origin; for many gradations of such were observed and in but a single case were omental adhesions present. If these considerations are well-founded it follows, to be sure, that the hypothesis which attributes the origin of accessory spleens to pre-natal traumata



is made still more improbable. For foetal life this assumption certainly seems out of the question for when one recalls the mechanical conditions in utero it is exceedingly difficult to realize how any trauma sufficient to disrupt the anlage of the spleen or the formed foetal organ, for that matter, would permit survival of the mother even if of the foetus itself. The writer has, for example, seen scores of isolated pregnant uteri containing foetuses in various stages of development, thrown with some force ten to twenty feet on the frozen plank floors of abattoirs, without ever having been able to detect a ruptured spleen. In fact, it is surprising how roughly excised unopened uteri can be handled with but slight or no damage to the foetus. Moreover, the liver ruptures far easier. Hence it has always seemed to me that any external force sufficiently strong and well localized to rupture the spleen of the foetus or to disturb it sufficiently to produce fragmentation must be wholly incompatible with survival of the maternal organism.

It will be recalled that Tizzoni (22) concluded that the new-formation of accessory spleens in the great and gastro-splenic omenta is always accompanied by special alterations of the main spleen as a result of which portions of the parenchyma cannot be bathed by the blood current. Tizzoni claimed that he always found a more or less circumscribed chronic interstitial splenitis present in these cases. He believed that this splenitis probably resulted from ruptures of the spleen which he thought to be especially prevalent in animals with a rapid gait such as the dog and horse and that the extent of the formation of accessory spleens was directly proportional to the lesion. Unfortunately for this conclusion scars and omental adhesions on the main spleen, are usually absent in cases of supernumerary spleens and the main spleens vary greatly in size and appearance both in the gross and microscopically. Some are large, soft and deeply red and others are small, firm and pale. The former contain a large amount of blood, many Malpighian follicles but may have large connective tissue trabeculae. The latter usually contain but little blood except in certain small areas, may have somewhat more evident trabeculae and few Malpighian follicles but no changes

or differences whatever were noted in the structure of the capsules. It is possible, to be sure, that these different characteristics of the spleen may be due to the same cause, one representing the initial and the other the final stage of the process but no evidence whatever was obtained suggesting that traumata constitute that cause. Moreover, the presence of a chronic interstitial splenitis was not confirmed nor any relation established between the probable fleetness of the animal and the occurrence of supernumerary spleens!

From the above observations and considerations it must be fully evident that the current conceptions regarding the origin of multiple or accessory spleens as found in man, are wholly inadequate or even fanciful when applied to cases in which such extremely large numbers of small isolated nodules are found present remote from a normal main spleen. Indeed, it is hardly conceivable, it seems to me, that numerous portions of an early embryonic anlage could lie dormant so long without undergoing regression even if not development, for many of the nodules found were so small that a section did not fill an oil immersion field. The structure not only of the smallest but of many others was exceedingly simple, as reference to figures 8 to 12 will show. All the sections shown here were taken from supernumerary spleens found in the great omentum. The structure as revealed by these specimens is quite similar to that of many hemal nodes although for reasons stated elsewhere<sup>5</sup> I am not willing to insist on their identity at present. All transitions in structure from such a nodule as shown in figure 8 of this plate to nodules containing Malpighian corpuseles and whose structure is indistinguishable from that of the main spleen can be found and it is these things which prompt one to consider a secondary origin. Nor do the other hypotheses considered by Schilling seem any more satisfactory explanations since very small apparently undeveloped, masses are found among others much larger and better developed. Hence, in conformity with the above hypothesis, it would seem to be necessary to assume that an extremely large

<sup>5</sup> Meyer, *Anat. Anz.*, Bd. 45, 1914.

number of widely disseminated fragments giving rise to independent anlagen were checked more or less permanently in their early development only to proceed independently or simultaneously at a later date or even that they were checked in different stages of their development. Indeed, from a careful histological study of many specimens taken from the great omenta of those dogs and cats which contained the very large numbers of accessory spleens above mentioned one can scarcely escape the impression which caused Tizzoni (19) to conclude that many of them are in process of formation. Moreover, it seems unlikely that they can be regarded as arising from portions of a single dissemination of disrupted anlage. The simplicity of structure of many of them lying remote from and foreign to the location of the original anlage would also seem to preclude such an origin. It is probable, to be sure, that the few cases of *lien succenturiatus*, and *lien lobatus* which were observed may have resulted from the separation of a small lobule or lobules of the main spleen by deep fissures as Henle (10) thought. Jolly, too, believed that his findings in infants supported this view which seems also to be confirmed by such cases as that reported by Helly (8) and others. However, it is interesting that Gegenbaur (5) rejected it and it is likely that such cases as that of Albrecht in which accessory spleens were scattered all over the peritoneum and in which several were also found on the serosa of the rectum and the case of Schilling in which a nodule taken for a spleen was found on the tip of an appendix epiploica of the descending colon, as well as those observed by Tizzoni in dogs probably cannot be accounted for in this way. Nor does the relation between accessory spleens and deformities suggested by such cases as those of Baille and Cruveilhier and of Otto with 7 and 23 spleens respectively, or of transformation of viscera, especially of the stomach, which was emphasized by Toldt (25) seem to be any better founded. Choronschitzky's (2) criticism regarding this matter and of Toldt's statement that the spleen develops in the right mesogastrium in case of transposition of the stomach, seem to be wholly justified. Besides, in the case of supernumerary spleens in man emphasis is usually also placed on the fact that the main spleen is smaller



than normal and that this decrease in size is somewhat directly proportional to the increase in number of the supernumerary spleens. In Albrecht's remarkable case, for example, the main spleen was only the size of a 'walnut' and directly contiguous with a group of accessory spleens at its inferior pole. In these dogs and cats containing these unprecedentedly large numbers of spleens there was no reduction in size of the main spleen. Nor was a gradation in size from the main organ through larger adjacent intermediate ones to the smallest of those farthest removed noticed. The structural characteristics of the smallest of these accessory spleens and their relation to the vascular system reminds one of Choronschitzsky's (2) statement that the earliest anlage of the spleen in the chick is in very intimate relationship to the venous system but has no such relation to the arteries. But these questions as well as the structure of accessory spleens, will be discussed elsewhere. Hence, I shall simply call attention to the fact that experimenters who have reported the formation of accessory spleens or hemal nodes after splenectomy without adequate information regarding the presence of these structures before operation have unwittingly drawn unreliable if not wholly erroneous conclusions and it is, for this reason alone, to be seriously doubted whether the alleged formation of accessory spleens or true hemal nodes—not hemorrhagic lymph nodes—after splenectomy rests on any other basis than that of uncontrolled and faulty experimentation. Moreover, it is interesting that Foa for similar and Schilling for entirely different reasons, also concluded that the experiments of Tizzoni, Griffini and Winoogradow led to no conclusive results and that according to Helly, Saltykow and Retterer both decided that the accessory spleens alleged to have been newly formed were present before operation.

Since true omental appendages quite incomparable to the fatty appendages of the colon in man, have been shown to occur, one cannot refrain from suggesting that the term 'appendices epiploicae' be applied to the former instead. This would be using the words with their true meaning and would rid the Basel terminology of another objectionable term. Besides, one can scarcely doubt that omental appendages similar to those here described

in dogs and cats occur also in man. Attention is also directed to the fact that since some of these omental appendages which may have a wholly different origin simulate supernumerary spleens so very closely but may not be such, the finding of pedunculated supernumerary spleens especially outside of the gastro-hepatic and greater omenta demands closer examination.

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## ERRATA

Hemal nodes in some carnivora and rodents. Studies on hemal nodes. III. Meyer, Anat. Anz., No. 12, Bd. 45, 1913.

Page 263, foot-note reference (2) read, Meyer, Anat. Rec., Phila., 1914; and similarly

Page 268, foot-note reference (1) Meyer, The hemolymph nodes of the sheep. Studies on hemal nodes. I. University Publications, Stanford University, 1914.



## THE NOMENCLATURE OF THE CARPAL BONES

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One of the most striking differences between the Basel Nomenclator Anatomicus and the terminology employed in English text-books lies in the names applied to the carpal bones. It seemed therefore that it might prove of some interest to trace back to its sources the terminology of the bones and to determine the origin of the differences that became established between the German usage (which was adopted) and that of French and English authors. The inaccessibility of certain works has prevented a perfect review of the literature, but I have nevertheless been able to reconstruct the history of the terminology with sufficient thoroughness to make the presentation of the results seem worth while, if only as matters of historical interest.

In contrast with the tarsal bones, whose names, with the exception of those of the three cuneiform bones,<sup>1</sup> date back to classical times, the carpals were late in receiving a definite terminology. By the older authors they were either described very superficially, without any attempt at a characterization of the individual bones, or else they were numbered, usually from the radial to the ulnar side and beginning with the radial bone of the proximal row. This was the mode of designation employed by Vesalius and was the mode in general use for over a century after the publication of the "De Fabricâ."

In 1653, however, definite names were for the first time applied to the bones by Michael Lyser, who was prosector to Thomas Bartholin in the anatomical theater at Copenhagen and published in that year the first edition of his "Culter anatomicus," a work that passed through five editions and whose scope is sufficiently indicated by its subtitle, "Methodus brevis, facilis ac perspicua

<sup>1</sup> The term cuneiform was first applied to these bones by Falloppius.

artificiose et compendiose humana incidendi cadavera; cum nonnullorum instrumentorum iconibus." The work is divided into five books, the fifth being devoted to the technique to be adopted in preparing and mounting skeletons, and it is in connection with this that a nomenclature for the carpal bones is suggested. It may be of interest to quote in full the passage in which the terminology is proposed:

Carpi ossa, si *Columbi* consilium arripueris, facile metacarpo annectere poteris: is enim in purificatione ligamenta hujus intacta dimittit, ut laboriosâ operâ ea iterum colligendi supersedere possit, quod et in pedii ossibus observare consuevit, scilicet taediosum nimis ista ossa in situm naturalem colligere, quod vix effectum dare licet, si non aliud sceleton exemplaris loco imitandum tibi proponas, ex quo positum ossium horum dignoscas: impossibile enim est oratione eum manifestare, cum propriis nominibus ossa ista careant. Tentabo tamen an aliquali descriptione, quo ordine conjungenda sint, indicare possim, impositis nominibus a forma eorum depromptis. Pollicis subjacet cubiformi simile, sed valde inaequalibus lateribus; trapezoides rectius diceres: Indici trapezium: Medius pro fundamento habet os omnium in carpo maximum et crassissimum, in postica parte capitulum obtinens: Annulari et minimo substat os unciforme, quia interius in manu unci in modum est incurvatum, huic adjacet in latere externo aliud ossiculum, cujus latera quatuor triangula conficiunt, cuneiforme dici posset; cui iterum adhaeret minus adhuc ossiculum pisi sativi magnitudine, parte ea, quae priori obicitur, depressum. Sex illa ossa ordine recensito connectenda. Ideoque singula bis acu pertundes, ac filum sicuti per summa metacarpi capita traduces: non tamen in recta linea conjunguntur, sed obliquè nonnihil et arcuatim. Bina adhuc supersunt ossa, quorum alterum *κοτυλοειδές* appello, obsinum, quo capitulum maximi ossis recipit: alterum lunatum nomino, quia sinum naetum est semilunarem, quo eidem capitulo occurrit.

From this it will be seen that Lyser termed the first or radial bone of the proximal row the cotyloid, the second lunatum, the third cuneiforme, while the fourth he merely describes as ossiculum magnitudine pisi sativi. In the distal row the first bone is named trapezoides, the second trapezium and the fourth unciforme, while the third receives no special designation but is described as "os maximum et crassissimum, in posticâ parte capitulum obtinens."

But notwithstanding the evident popularity of Lyser's book, it was many years before his carpal terminology began to find

favor among anatomists. For so far as I have been able to ascertain, it was not until 1726 that it received any definite recognition, the anatomical text-books published before that date, and to some extent even after it, continuing to adhere to the numerical designation of the bones, or else failing to consider them individually. Thus Bidloo (1685) adopts the former plan, and Cowper (1698) in his reissue of Bidloo's plates with an English text, naturally does the same, while Verheyen (1699) follows the latter, as do also Heister (1717) in his "Compendium" and the English Cheselden in his "Anatomie of humane bodies" (1713). Both these works passed through numerous editions, that of Cheselden appearing in sixteen and that of Heister, the prototype of the modern quiz-compend, as many as twelve Latin editions, as well as in five German, four French, two English and a Russian translation. From their popularity it may be presumed that they represent fairly accurately the scope of anatomical instruction in their day, but a hint at a knowledge of the fact that names had been bestowed upon the bones is to be found only in the second edition of the "Compendium" (1727), in which in a footnote the author remarks, "There are some who give names to the ossicles of the carpus, a thing which I regard as unnecessary and useless (*supervacaneum et inutile*). If, however, they are to be distinguished and named, I think it should be by number." This, however, is not necessarily a reference to Lyser's work, since it followed the publication (1726) of other works in which a definite nomenclature was adopted.

But the lack of acceptance of Lyser's suggestions is shown even more clearly in the fact that works dealing exclusively with osteology, published between 1653 and 1733, make no mention of his nomenclature. Thus, in the "Osteologia corporis humani" of Senguerius (1662) the carpal bones are dismissed with little more than the statement that there are "eight bones, vary varied in form," and Palfijn of Ghent in his osteology, written in the Dutch language (1702), gives a very superficial account of them without names and in his "Anatomie du corps humain," published at Paris in 1726, they are numbered from the ulnar side, beginning with the distal row, and but three bones are assigned



to the proximal row, the pisiform being mentioned as the eighth bone "*hors du rang*." Lancisi in his editions of the "*Tabulae anatomicae*" of Eustachius (1714, 1722) gives no designations to the bones and in the elaborately illustrated "*Osteographia*" of Cheselden (1733) they are also unnamed. Several other osteological treatises, of this period, such as those of de Pauw (1615) and Guillemeau (1618), I have not been able to consult, but from what has been said above it seems clear that Lyser's suggestions had been rather barren of results until 1726, even although his book was in sufficient demand to warrant the publication of its fifth edition in 1731.

In 1726, however, two osteologies appeared which have had an important influence on the nomenclature of the carpal bones. One of these was "*The anatomy of the humane bones*," by Alexander Monro, the first of that name in the University of Edinburgh, in which the description of the carpus is introduced as follows:

Carpus is composed of eight, small spongy bones situated at the upper part of the Hand. Each of these Bones I shall describe with Lyserus under a proper name, taken from their figure because the method of ranging them by Numbers, leaves Anatomists too much Liberty to debate very idly, which ought to be preferred to the first Number: or, which is worse, several, without explaining the order they observe, differently apply the same Numbers, and so confound their Readers' ideas.

The names adopted by Monro are, with one slight exception, those that have become familiar to students of English textbooks and are as follows, alternative names, which he assigns to footnotes, being placed within brackets: scaphoides (*naviculare*), lunare, cuneiforme, pisiforme (*cartilaginosum*), trapezia, Trapezoides, magnum, unciforme.

It will be seen from this list that while professing to follow Lyser, Monro has departed from his suggestions in certain respects. Thus he substitutes for Lyser's *cotyloides* the more familiar term *scaphoid*, giving the Latin equivalent as an alternative; instead of *lunatum* he uses *lunare*; a definite name is given to the pisiform with an alternative in *cartilaginosum*: the Lyserian

names for the two radial bones of the distal row are transposed; and the third bone of that row is given a definite name, magnum however being used instead of the superlative maximum found in Lyser's description. Monro gives no explanation of his modification of Lyser's terms, the transposition of trapezoid and trapezium being especially noteworthy; possibly as Blumenbach has suggested, the original source was not consulted at the time of writing, the terms being applied from memory. But, however that may be, it was Monro's application of trapezium and trapezoid and not Lyser's that was adopted by later writers.

The other work of 1726 referred to above was the "*De ossibus corporis humani*" of B. S. Albinus in which an almost entirely different set of terms is used, the bones, in the order in which they are taken above, being named: naviculare, lunatum, triquetrum, subrotundum, multangulum majus, multangulum minus, capitatum and cuneiforme. This gives us the source of the B. N. A. terms, the only difference being in the use of subrotundum for the pisiform and cuneiforme for the hamatum. I have not been able to examine Albinus' "*Tabulae sceleti et musculorum corporis humani*" (1747), but in his earliest edition of the "*Tabulae anatomicae*" of Eustachius (1744) the terms used are the same as those given above.

We have thus in these works of Monro and Albinus the source of the usages adopted by English and German anatomists respectively for the nomenclature of the carpal bones. The French usage appears to date back directly to Winslow, that curious compound of keen observation and mysticism; the son of a Danish clergyman, destined to follow his father's profession, but later relinquishing theology for medicine and coming to Paris where he became a convert to Catholicism under the tutelage of Bossuet, the Bishop of Meaux, and eventually succeeded Hanault in the chair of Anatomy and Surgery in the Jardin du Roi. The names he employed in his "*Exposition anatomique de la structure du corps humain*" (1732) are based on those used by Monro. Winslow professes to quote Lyser, but in reality the terms he gives are Monro's; thus he says:

Lyserus a donné des noms à chacun de ces os. Il a nommé du premier Rang le premier Os Scaphoïde ou Naviculaire; le second Os Lunaire; le troisième Os cunéiforme; le quatrième qui est hors du Rang os pisiforme ou Lenticulaire. Dans le second Rang il a nommé le premier os Tra-pèze; le second os Trapezoïde; le troisième le Grand os et le quatrième l'os Crochu ou Unciforme.

In the description of the individual bones, however, he modifies certain of these terms, substituting 'semilunaire' for lunaire and 'orbiculaire' for pisiform or lenticular and suggesting the appropriateness of the term 'pyramidal' for the trapezoid. Tarin in his "Osteographia" (1753) substitutes naviculare for scaphoïde and cuboides for cuneiforme and employs the semilunare of Winslow instead of lunaire, but otherwise he follows the terminology of Monro, and Sabatier in his "Traité complet d'anatomie," which had considerable vogue, follows Monro exactly, except that he uses semilunaire instead of lunaire. So too Bichât in his "Traité d'anatomie descriptive" (1801). The "Traité d'ostéologie" of Bertin (Paris, 1754) I have not seen.

It is unnecessary to trace in detail the further history of the terms in Great Britain and Germany. For the former it is sufficient to state that Monro's terms were quickly adopted, although later, probably owing to French influence, semilunare began to supplant lunaire. In German text-books towards the close of the eighteenth century it became the custom to employ the vernacular in naming the various bones, these appearing as Kahn-bein, Mondbein, etc., the terms employed being, however, in all cases translations of the Latin ones of Albinus. But the synonymy is always given more or less fully, and sometimes new synonyms were suggested. Thus Soemmerring (1791) suggests tri-angulare as a synonym for das drei-eckige Bein, lentiforme for das runde Bein, rhomboides for das grosse vieleckige Bein and hamatum for the Hackenbein, this last term later replacing the cuneiform of Albinus, probably from the fact that this name was also a synonym for das dreieckige Bein. It may also be mentioned that Hildebrandt (3d ed., 1804) gives pyramidale as one of the synonyms for the cuneiform and os extra ordinem for the pisiform, latinizing the expression os (hors) du rang applied to it



by Sabatier (3d ed., 1791) and before him by Palfijn (1726). It is worthy of note, however, that while the German authors thus generally adopted the terminology of Albinus, Jacob Henle, one of the greatest anatomists that the country has produced, preferred a set of terms more nearly resembling those of Monro. His terms (3d ed., 1871) are as follows: Kahnbein, os scaphoideum; Mondbein, os lunatum; Pyramidenbein, os pyramidale; Erbsenbein, os pisiforme; Trapezbein, os trapezium; Trapezoidbein, os trapezoides; Kopfbein, os capitatum; Hakenbein, os hamatum.

From what has been said it is evident that the terms for the carpal bones employed in the B. N. A. are open to criticism on several counts. They do not represent the usage of the majority of those who are obliged to employ such terms; if we may allow some weight to priority, they are with one exception antedated by the Lyserian names; and two of them, *multangulum majus* and *minus*, are cumbersome and, being binominals, are little suited for the formation of derivative words. It is unfortunate that the Commission did not see fit to adopt the nomenclature used by Henle, substituting perhaps *triquetrum* for his *pyramidale*, cuneiform being thus left for application solely to the tarsal bones. We should then have had a set of terms of convenient brevity and form and recognizing the historical development of the terminology.

The following is a list of the synonyms of the carpal bones, so far as I have been able to trace them, together with the name of the author who first used them and the date. In certain cases I have not been able to determine the date exactly, owing to the fact that I have had access only to a later edition of the work in which they occur; in such cases the number of the edition consulted is inserted before the date. The bones are arranged in the usual order.

Cotyloides, Lyser (1653); scaphoides, Monro (1726); naviculare, Albinus (1726).

Lunatum, Lyser (1653); lunare, Monro (1726); semilunare, Winslow (1732).

Cuneiforme, Lyser (1653); triquetrum, Albinus (1726); cuboides, Tarin (1753); triangulare, Soemmerring (1791); pyramidale, Hildebrandt (3d ed., 1804).

Pisiforme, Monro (1726); cartilagosum, Monro (1726); subrotundum, Albinus (1726); os hors du rang, Palfijn (1726); orbiculare, Winslow (1732); lenticulare, Winslow (1732); lentiforme, Soemmerring (1791); os extra ordinem, Hildebrandt (3d ed., 1804); rectum Kirby (in Monro 4th ed., 1828).

Trapezoides, Lyser (1653); cubiforme, Lyser (1653); trapezium, Monro (1726); multangulum majus, Albinus (1726); rhomboides, Soemmerring (1791); rhomboideus, Hildebrandt (3d ed., 1804).

Trapezium, Lyser (1653); trapezoides, Monro (1726); multangulum minus, Albinus (1726); pyramidale, Winslow (1732); magnum, Monro (1726); capitatum, Albinus (1726).

Unciforme, Lyser (1653); cuneiforme, Albinus (1726); hamatum, Soemmerring (1791).

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1733 *Osteographia or the anatomy of the bones*, London. Later editions appeared in 1811, 1813, 1822.
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## BOOK REVIEW

PRACTICAL ANATOMY: THE STUDENT'S DISSECTING MANUAL. By F. G. Parsons, F. R. C. S. Eng., and William Wright, M.B., D.Sc., F.R.C.S.Eng. In two volumes, New York: Longmans, Green and Company; London: Edward Arnold. 1912.

The authors present a two-volume dissecting manual for use by students in their practical work in the anatomical laboratory. They wisely, in those instances in which they have used it, have made secondary the Basle nomenclature, which at the present time is impractical for general student use.

Following a brief introduction, there appear in volume 1 some general hints on dissecting, and the authors suggest various appliances that the medical student upon beginning dissection should have in his kit. Among these are a "strop, on which he should strop his knife every ten minutes"—thus wisely emphasizing the necessity for sharp knives,—“knitting needles,” “crochet hooks,” and various other homely articles which will be found of real value to those of mechanical temperament and ingenious mind.

The authors advise drawing the parts at various stages of the dissection, a commendable but hardly a practical plan, for the reason that the time now allotted to dissecting in a medical course does not permit of this desirable procedure. No mention is made of the action of the various muscles, nor is any attempt made to teach the function of any of the nerves. While this would encroach upon the domain of physiology, it would be a valuable addendum to the volumes. The teaching of structure and function should not be separated too widely.

The general plan of dissection is in the main the same as that found in other present-day dissecting manuals.

In volume 1 the dissection of the face precedes that of the neck: this is a procedure which has not been found by all to be advantageous. In this section the description of the fascia cervicalis is brief and misleading and its importance is not noted, thus minimizing the real value of an accurate conception of this structure.

On page 61, volume 1, occurs a true 'Briticism.' The analogy is drawn here on the one hand between the relationship of the lacunae laterales to the superior longitudinal sinus and on the other that of the 'Norfolk Broads' to their rivers. While this is a privilege of authorship, at the same time it is exerted at the expense of the geographically uninformed non-British student.

The parathyroids (page 97, vol. 1) are described as 'embedded' in the thyroid gland and as seldom seen in the dissecting-room, while no mention is made of their blood supply; the inadequacy of this descrip-

tion is obvious. The importance of the *plica triangularis* of the tonsil is lost in the description on page 192, volume 1, this structure being described meagerly but not named. The authors incorrectly speak of a fifth ventricle on page 326, volume 1. The importance of the osteofascial compartments of the thigh is not mentioned, and here again the text is lacking. The student certainly is unable to gain any clear knowledge of femoral hernia from the brief and unsatisfactory attempt to explain it on page 381.

Volume 2 continues the same discrepancies and antiquated descriptions which characterize the first half of this work. The illustrations are of the same poor quality as in volume 1, those depicting the interior of the heart (pages 19 and 21), for example, being very crude. The description of the mediastinum is complete and possesses a definiteness which is largely lacking throughout both volumes. The perineum is accurately described but so poorly illustrated that the dissection of this very difficult and obscure region is rendered less clear for the student than is the case in other dissecting manuals.

The illustrations in both volumes are largely poor sketches and many are misleading and wholly out of proportion. On pages 77, 80, 90 and 92, volume 1, the diagrams are grotesque, and in many instances they are quite difficult of comprehension—inexcusable faults in any book, but especially in a laboratory manual. The type is clear and the paper good. In the experience of the reviewer, the young and inexperienced student would welcome and be helped by a clearer blocking out of directions for dissecting. Such information is better imparted and more easily found if a different font of type is used for this purpose, but in this book no such plan is followed; the same type is used throughout.

This manual is certainly inferior to its predecessors, including both those of English and those of American authorship. The chapters appear to be hastily written, and they bear the personal equation of authorship to an injudicious and unscientific degree. The reviewer feels that this work in its present form can not be recommended to the student whose limited time for dissection demands the best of assistance; and furthermore, it fails to equal in many and to surpass in any respect other volumes of a like nature now available.

G. F.



## THE NERVUS TERMINALIS IN MAN AND MAMMALS<sup>1</sup>

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NINE FIGURES

It is over nineteen years since Pinkus ('94) first called attention to a 'new nerve' attached to the telencephalon of *Protopterus*, and thirty-five years since the first record of this nerve having been seen in a shark (Fritsch '78). The forms in which this nerve has now been recorded and its chief characters have been briefly summarized in the writer's previous communication ('13). In that paper the existence of a true *nervus terminalis* in human and certain mammalian embryos was clearly established. At the same time McCotter ('13) pointed out the existence of the nerve in the adult cat and dog. Huber and Guild ('13) have since studied the peripheral relations of the nerve in the rabbit in late foetal stages and during the first six days after birth.

The purpose of the present note is to call attention to the presence of the *nervus terminalis* in certain other adult mammals in the hope that a larger number of workers may undertake the study of its central and peripheral relations. At the present time it is clear that at least a part of the nerve is distributed to the mucosa of the nasal sac and in mammals accompanies the vomero-nasal nerve. A part of the nerve, however, in mammals clearly goes beyond the limits of the vomero-nasal organ. In the rabbit it spreads over a rather wide area of the nasal septum (Huber and Guild). The nerve is usually accompanied by ganglion cells, which Brookover ('10) believed to be sympathetic in character. Huber and Guild incline to the same conclusion. Although the central relations of the nerve have been studied by special methods by Herrick ('09), Sheldon ('09) and McKibben

<sup>1</sup>Neurological Studies, University of Minnesota, no. 19, November 15, 1913.

(11) it is still not known whether its fibers are all afferent, or whether some or all of the fibers arise from cells within the brain. In the latter case they might be considered preganglionic fibers of the sympathetic system. The present state of our knowledge suggests the probable presence of both afferent and efferent components in the series of vertebrates. The wide-spread presence of the nerve in adult mammals, including man, should add interest to the study of its relations.

*The pig.* In my previous communication it was stated that the nerve had not been seen in 73 and 90 mm. pigs. Since then it has been found by dissection in numerous pig fetuses ranging from 50 mm. to full term. The brain of the adult pig has not been examined.

*The horse.* Figure 1 shows the proximal portion of the right nervus terminalis in the brain of an adult. The figure shows a small portion of the basal surface of the brain between the olfactory trigon and the median fissure. A part of the anterior cerebral artery is seen in the right hand part of the figure. The plexus of small vessels lies immediately upon the brain substance, the nervus terminalis lies outside of them and is in turn covered by the pia. The nerve has about fourteen rootlets which enter the brain along the rostral and medial border of the medial olfactory tract. The rootlets unite by twos and threes and eventually form a common nerve trunk. Upon the largest one of three main roots into which the rootlets unite, as seen in the figure, there is an obvious ganglion. The nerve trunk extends forward nearly parallel with the olfactory peduncle to a point opposite the rostral border of the olfactory bulb, where it is lodged in the pial septum between the hemispheres. Here the nerve had been cut off in removing the brain from the skull.

The nerve of this side was removed after drawing and cut into three pieces for staining. The distal piece was treated with vom Rath's picro-osmo-palatino-acetic mixture, cleared in cedar oil and mounted in damar. It contains three fairly well medullated fibers and seven or eight fibers which were lightly and irregularly blackened. The middle piece was stained in a mixture of nigrosin and acid fuchsin but a differential staining of nerve fibers

and connective tissue was not obtained. The proximal piece included a part but not all of the ganglion seen in figure 1. The piece was stained in neutral red. A number of cells were found scattered along this piece and the portion of the ganglion consisted of about twenty closely packed cells varying in size. All



Fig. 1 Root of the nervus terminalis in the horse, right side. Description in text.

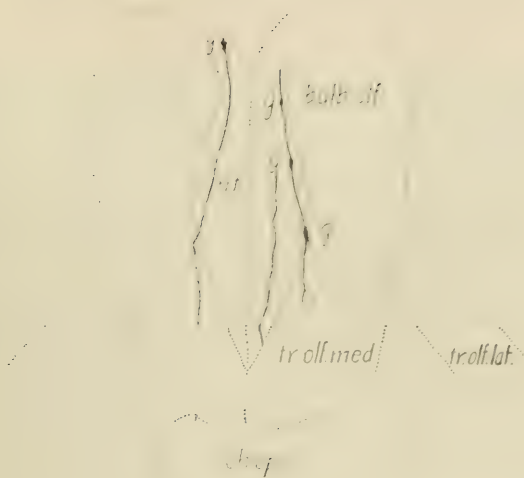
the cells had large nuclei with prominent nucleoli. Although the Nissl bodies were not clearly stained, owing to unsatisfactory fixation, there is no doubt that the cells are nerve cells. Two nerve cells were seen also in the piece stained by nigrosin.

The nerve on the left side of this brain is similar to this although it differs in the number and arrangement of rootlets.

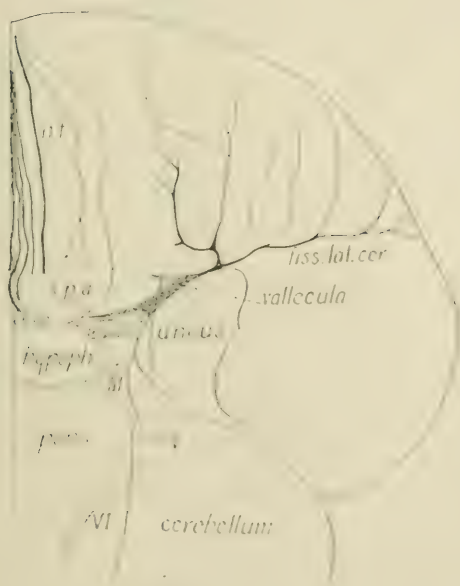


*The sheep.* Three brains of the adult sheep have been examined. The nerve was not found in the first but was present in the other two. In one of these brains (fig. 2) there was a single strand on the right side and two strands on the left. One of the latter strands presented three conspicuous ganglion-like enlargements. Upon staining and mounting these proved to be true ganglia. The nerve of the right side contained single ganglion cells scattered along its course, two collections of six or eight cells each and a ganglion at its distal end larger than any one of the three on the left side. A piece of the left nerve, treated in vom Rath's fluid, showed a single lightly medullated fiber.

*The porpoise.* I am indebted to Mr. W. F. Allen of this laboratory for the brain of a porpoise (*Phocaena*) preserved in Bouin's fluid. The brain of the porpoise has a very broad, rounded frontal lobe (fig. 3), the optic tracts diverge very widely and the anterior perforated space is greatly elongated from side to side. In the absence of the olfactory bulb and peduncle the topographical relations in this part of the brain must be based chiefly on the extent of the anterior perforated space. Upon the basal aspect of the frontal lobe there are seen beneath the pia seven slender strands which converge forward to a point corresponding as nearly as may be judged to the point at which the *nervus terminalis* was cut off in the horse's brain. Here likewise the nerve had been cut in removing the brain. Proximally the strands enter the brain over a wide area. The most lateral one enters the lateral part of the anterior perforated space. The most medial strand runs along the median fissure and bends up on the medial surface to penetrate the brain in the *fissura prima* on this medial surface (fig. 4). Two strands follow the anterior cerebral artery in the median fissure and bend laterad with it almost in contact with the rostral surface of the optic chiasma and enter the brain in the depth of the *fissura prima* on the basal aspect. The nerves of the left side have been described and drawn; those of the right side have a similar arrangement. The nerves are relatively larger than in the horse and are more closely applied to the brain surface throughout their course. They differ also in that the rootlets are flattened strands which run for a longer distance before uniting.



2



3

Fig. 2 Nervus terminalis in the sheep; *g*, ganglia; *ch.op.*, optic chiasma.

Fig. 3 Basal aspect of the brain of the porpoise; *s.p.a.*, substantia perforata anterior. The broken line bounding this rostrally marks a small sulcus occupied by a blood vessel. Three of the rootlets penetrate the brain beneath this vessel. *X* indicates point at which a root was broken in dissection.

*The monkey.* The brain of *Macacus rhesus* and that of *M. cynomolgus* have been examined. Both show the nervus terminalis in characteristic form (fig. 5). Rostrally several nerve strands converge on the orbital surface of the frontal lobe (gyrus rectus) between the olfactory bulb and the median fissure and are cut off opposite the anterior end of the bulb. Traced proximally these strands diverge over the surface of the gyrus rectus and bend down into the fissura prima. The number of rootlets is greater in the rhesus, but otherwise the arrangement is essentially the same. The most mesial and thickest strand was removed from the rhesus brain, treated with vom Rath's fluid and stained with carmalum. The strand proved to be so compact that it could not be teased out with needles and only its proximal and distal portions could be examined satisfactorily. The rootlets contained no medullated fibers but in the distal one-fourth of the nerve medullated fibers began to appear and increased until there were fourteen to be seen at the distal end of the portion mounted. Near the distal end was seen a single large, typical ganglion cell. Several small cells in both the proximal and distal ends presented the appearance of nerve cells.

*Man.* I have examined a foetus of the fifth month, one of seven months, one at full term, a baby of four months and fourteen adult brains.

The five-months foetus had been long in Zenker's fluid or other bichromate solution and was very brittle when it came into my hands. On removing the right hemisphere the nerve of the left side was readily seen as a good-sized whitish strand extending from the fissura prima forward parallel with the olfactory peduncle toward the septum medial to the bulb. The condition of the material made it impossible to follow the nerve peripherally.

Fig. 4 Medial aspect of part of the left half of the brain of the porpoise. The dotted line *m-b* is the line of meeting of the medial and basal surfaces of the frontal lobe. The dotted line below is the profile of the rounded basal surface of the frontal lobe. The three more medial roots are shown. The most medial one enters the brain below the anterior commissure (*c.a.*); *c.f.*, fornix.

Fig. 5 Nervus terminalis in the monkey. The nerves of the right side of *Macacus rhesus* and those of the left side of *M. cynomolgus* are drawn; *f.p.*, fissura prima.





The seven-months foetus had been in formalin for at least six years and had not been fresh enough for histological study when preserved. The brain was rather soft and the tissues tough, so that the attempt to trace the nerve into the nose had to be given up. The brain was carefully removed and upon examination under the Greenough binocular, two transparent nerve strands were seen (fig. 6) upon the orbital surface of the gyrus rectus which were cut off opposite the anterior end of the olfactory bulb as in the forms above described. The apparent change in the position of the nerve since the five month stage is due to the

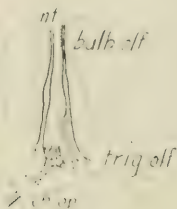


Fig. 6 Seven months human foetus, basal surface of the frontal lobes showing the nervus terminalis.

rapid development of the frontal lobe, which has expanded mesad producing a gyrus rectus medial to the olfactory peduncle. The pre-chiasmatic space is filled with a gossamer-like fibrous tissue which had to be removed patiently in order to follow the strands to their point of entering the brain. On the left side one of the strands pierced the rostral border of the medial olfactory tract near the trigon. The other strand divided into two rootlets which ran deeper into the fissura prima (fig. 6). On the right side the nerves were not fully dissected, the anterior cerebral artery being left in position to show the relations.

In the full-term foetus the attempt was made to trace the peripheral course of the nerve. The dissection was made from the face in order to expose the orbital surface of the frontal lobe. The

nerve of the left side was found on the gyrus rectus as in other cases. It was a single thick strand readily visible to the naked eye. Traced forward it entered the median fissure opposite the anterior end of the olfactory bulb. Curving somewhat dorsad in the fissure the nerve makes a gentle curve ventrad again and at the same time leaves the surface of the brain, with which it is in contact, and enters the pia mater. At the same time the nerve divides into several strands which flatten out like a fan. These thin flat strands pierce the pia and enter the tissue of the cribriform plate close to the median plane among the most anterior strands of the olfactory nerve. Here the connective tissue was so tough, owing to the formalin preservation, that the thin strands could not be followed far. Some of them were followed without doubt into the septum, and some appeared to go toward the lateral wall of the nasal chamber, but this was uncertain. The point at which these strands pierce the pia mater is the place where the nerve is cut off when a brain is removed from the skull. The fact that at this point the nerves in the adult brains dissected were either within or very near to the median fissure and imbedded in the pia, suggested the possibility that the nerves might be distributed to the meninges, but the dissection of this specimen was carried far enough to show conclusively that they go down into the septum, and to explain their position in the adult brain.

The brain of a baby of four months showed two strands on the left and three on the right. On both sides the roots entered the brain beneath the medial and rostral border of the medial olfactory tract. The nerve of the left side was stained and mounted, but no ganglion cells were found.

Fourteen adult human brains have been examined and the nervus terminalis found in all. In most cases the nerve is visible under the lowest power of the Greenough binocular without any dissection. It is only necessary that the pia mater shall be intact in the region between the olfactory peduncles and rostral to the optic chiasma. In only one of the fourteen brains was the nerve so small as to be at all difficult to follow. The nerve strands lie just beneath the pia and are visible through it because slightly



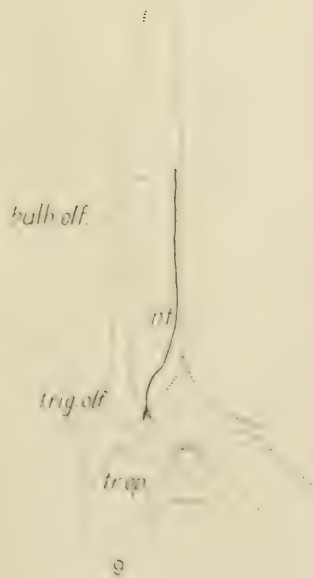
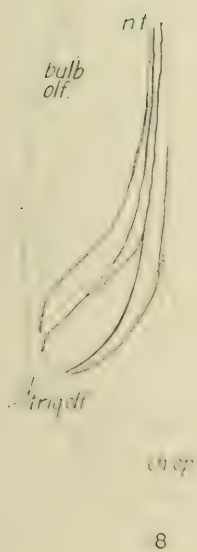
more whitish opaque than the pia. They are distinguished from small blood vessels because the vessels, even when apparently empty, have a slightly yellowish color. Moreover, small blood vessels are readily traced to the larger ones from which they arise. It is more difficult to distinguish the *nervus terminalis* from thin bands of connective tissue which lie in or beneath the pia. Most of the connective tissue strands in this region are inserted in the thick tissue surrounding the anterior cerebral artery or some of its branches in the median fissure, from which they stretch obliquely forward and laterad. The connective tissue strands can be detected by pulling this way and that upon the pia with fine forceps. The strands of connective tissue will be distorted and the appearance of strands will be produced parallel with the direction of tension, while the nerve strands, if present in the area pulled upon, will not be distorted, nor obscured. When the pia mater is pricked through, lifted up with forceps and dissected away it is found that the nerve strands lie beneath the pia but are attached to it more or less closely. The attachment is by means of slender connective tissue strands and in some instances by small bands which pass beneath the nerve in the form of loops or straps. In some cases the length of the nerve was drawn through one of these loops in order to free it from the pia. With a little care the nerve is separated from the pia and a few minutes suffice to expose the nerve through the greater part of its intracranial course. The dissection of the roots where they bend around the heel-like convexity of the gyrus rectus just in front of the optic chiasma requires more care, especially when the nerve divides into several slender rootlets.

Figures 7, 8 and 9 show the course of the nerve in three adult brains. It runs over the orbital surface of the gyrus rectus and

Fig. 7 Part of the basal aspect of the left frontal lobe in the adult human brain. The blood vessel is a small vein greatly distended with blood. X marks a root broken in dissecting.

Fig. 8 Basal aspect of the right frontal lobe in the adult human brain. Anas-tomosis of rootlets.

Fig. 9 Similar to figure 8. Nervus terminalis, a single strand with five rootlets on the surface of the medial olfactory tract.



enters the median fissure or lies near it at the level of the anterior end of the olfactory bulb, where the nerve is cut off in removing the brain. As seen in the figures, the nerve in these cases consisted of one, two, three or four strands. In one case (fig. 7) one of the strands divided into three strands which again united into one. In another case (fig. 8) a slender strand crossed obliquely from one of the main strands to another. It is entirely possible that slender strands would be overlooked or destroyed in dissecting and two or three minute strands were seen which are not included in the drawings. In the fourteen brains the type of nerve most frequently met with is that shown in figure 8. In one case the subdivision and reuniting of strands seen in figure 7 was present, together with a large number of very slender strands which ran along the medial border of the olfactory peduncle and bulb. When the nerve consists of a single strand, as in figure 9, it is large enough to be seen and dissected without the use of a lens. In one case it appeared larger than some of the rootlets of the IX and X nerves; but, being a broad thin band, it probably contains fewer fibers than those rootlets.

The point of attachment of the roots to the brain varies considerably. In all cases thus far examined it is in the region of the basal end of the fissura prima, either upon or in front of or behind the medial olfactory tract. In figure 8 the roots are seen converging toward the olfactory trigon, where they seemed to dip beneath the anterior and medial border of the olfactory tract. In figure 9 the single root divides into five rootlets which pierce the medial olfactory tract. The two roots of the other side of this brain had the same position. Two of the three roots in figure 7 have nearly the same position but one of them passes farther toward the median plane.

In position these strands correspond very closely to the *nervus terminalis* of lower animals and of human embryos previously described. Proof of the nervous character of the strands was sought, however, by removing and staining some of them. In one brain the nerve was found on only the right side. Its two rootlets entered the brain just at the rostral border of the medial olfactory tract where that bends from the orbital to the medial



surface. The roots were pulled out and the entire nerve was stained in neutral red and mounted in damar. It consists of some fifteen or sixteen small bundles of non-medullated fibers and has imbedded in its course about twelve ganglion cells. These cells occur singly or in twos. There is no larger collection. Each cell has a large nucleus and prominent nucleolus and stains deeply with neutral red. Unfortunately the material was not fresh enough to give a good stain of the Nissl substance.

The nerve on the right side of the brain from which figure 9 was taken was removed and stained as was also the left nerve of the four months baby brain, but no nerve cells were found in either. From the brain shown in figure 8 the nerve of the left side was stained in neutral red, while that of the right side was treated with vom Rath's fluid and afterward stained with carmalum. Neither medullated fibers nor nerve cells were found in this case. The largest nerve found was treated in the same way and teased out carefully. After clearing and mounting in damar there were found four ganglion cells surrounded by numerous sheath-cell nuclei. Two cells were in the middle part of the nerve, two at the distal extremity. No medullated fibers were found. Failure to find ganglion cells in some of the other cases may have been due to the fact that the nerves were not as well teased out.

From the above facts it appears that a nerve corresponding to the nervus terminalis of lower vertebrates exists in adult man and several other mammals. This nerve contains some medullated fibers at least in the sheep, horse and monkey, and in the monkey these fibers increase in number distally. In the sheep, horse, monkey and man the nerve contains ganglion cells in at least some individuals. In the sheep and horse there are distinct ganglionic enlargements of the nerve. The failure to find ganglion cells in the other cases here reported has little significance, since only the intra-pial course of the nerve could be examined. The probability that ganglion cells would be situated farther distally is suggested by the condition in the rabbit (Huber and Guild) and by the presence of a large ganglion in the sheep (fig. 2) just at the point where the nerve pierces the pia, rostral to the olfactory bulb. The presence and large size of the nervus termi-

nalis in the porpoise serves to emphasize its independence of the olfactory nerve, which has been abundantly established by previous work.

The nerve has usually been regarded as a vestigial structure, but it is an interesting fact that it is larger in man than in many fishes and amphibians. The question suggests itself, for how many million years has the nerve persisted in a vestigial condition? Further studies are desirable upon the central relations of the nervus terminalis and upon its structure and distribution in the nasal septum.

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## A NOTE ON THE CIRCULATION OF THE CORNU AMMONIS

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### TWO FIGURES

In the course of a study of the distribution of the lesions of general paralysis<sup>1</sup> some injection experiments were carried out to determine how great a factor the blood from the carotid system of cerebral vessels plays in the supply of the cornu ammonis and its associated structures, and the results seem worthy of record.

Six brains in all were injected. Four of these received an injection of a simple aqueous solution of a dye, and two were injected with colored gelatin masses. Immediately on removal of the brain from the body, cannulae were inserted into the basilar and two carotid arteries and normal salt solution from a 5-gallon bottle was allowed to run through the vessels by means of a gravity syphon and under a comparatively low pressure. In one each of the aqueous and gelatine group, the injection was made through the carotid system without opposition from the basilar group. In the others, Beevor's method of simultaneous injection into both trunks under equal pressure was employed.

It frequently happened that the carotid arteries were cut too close to the brain on removal, to permit the introduction of a cannula and in this circumstance the cannula was inserted into either the anterior or middle cerebral and the carotid opening tied, as well as all branches of the carotid system except the anterior choroid. The posterior communicating artery was tied in all instances. In one brain of the six the anterior carotid artery arose from the posterior communicating.

The path of communication from the carotid system to the hippocampal region is by a branch of the anterior choroid artery

<sup>1</sup> To appear in the American Journal of Insanity.



which has been found to be of constant occurrence, though variable in size, in both hemispheres of all of a series of twenty-five brains examined, and which reaches the uncus hippocampus to which it gives cortical branches and then, as is apparent in the injected specimens, deeply penetrates the white matter of the cornu for a variable distance.

In all instances there was definite staining by the color carried through the anterior choroid artery of the cortex of the uncus hippocampi and of the central white core of the cornu and while the extent of this coloration varied somewhat in the different specimens of the opposed series, in none of them was it as great as in the brains in which the injection was made unopposed through the anterior choroid.

This discrepancy in the size of the injected area may of course be due to individual variations in the supply which happened to be of greater importance in the two with unbalanced injections, but the explanation also seems tenable that in the unopposed injections the mass penetrated into portions of the field supplied by the posterior cerebrals by means of anastomotic pathways. This type of anastomosis of neighboring cortical fields has been described in areas of the neopallium, that is, between branches of the anterior and middle cerebrals on the dome where the circulation is by no means so strictly endarterial as is the case in the basal ganglia, and the same may be true in this archipallial region.

The accompanying illustrations show the distribution of an unopposed injection in a brain obtained from a case of general paresis about three hours after death. Some clotting had occurred in the large vessels before washing, but the smaller vessels give little or no evidence of plugging.

Figure 1 shows the right temporal pole and hippocampal region with the planes of section and the distribution of the injection mass in these planes. In this case the injection was made through the anterior choroid by way of the anterior cerebral and without a balancing posterior cerebral injection. The posterior communicating artery was tied near its posterior cerebral end and some gelatin found its way into the brain stem through branches of this vessel.

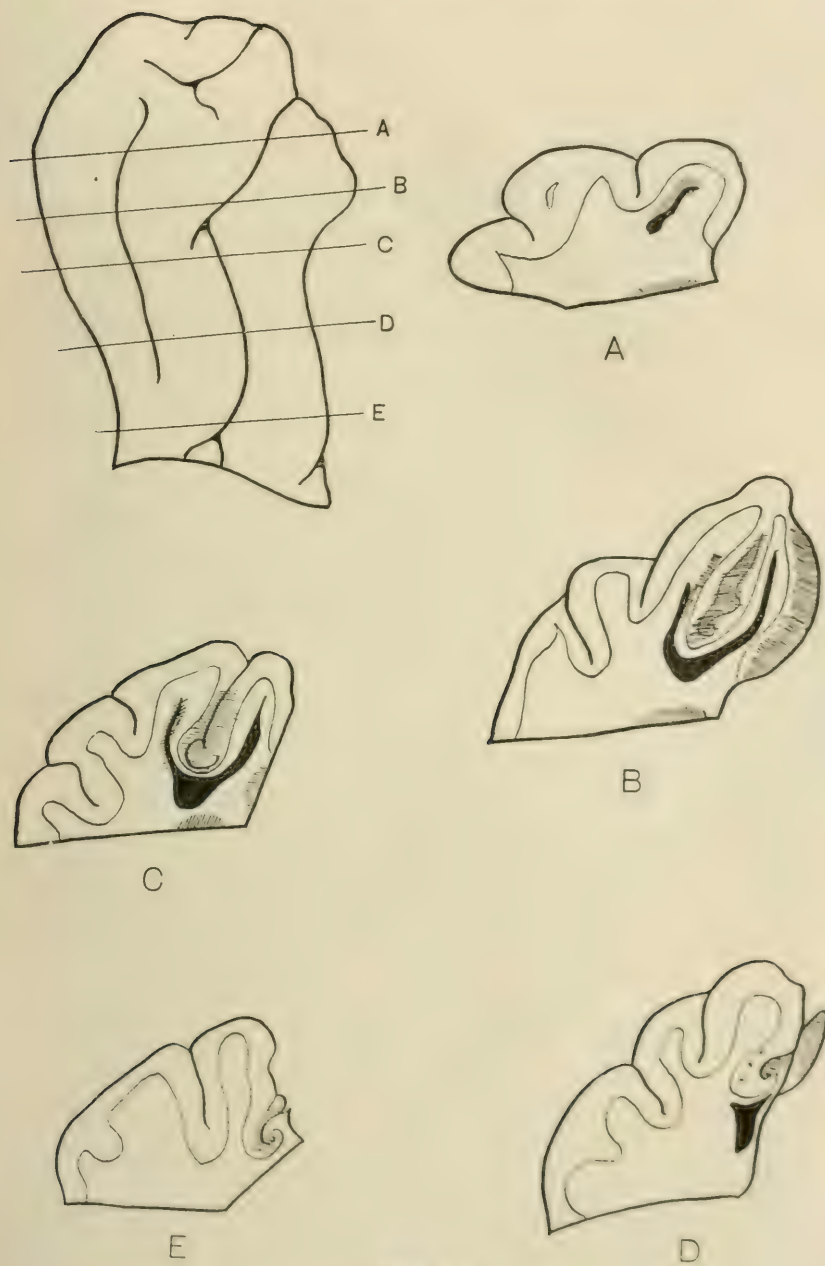


Figure 1



Figure 2

Figure 2 is a projection drawing of a paraffin section from the specimen shown in figure 1 and illustrates in the larger drawing the relation of the injected material to the various cell and fiber layers and in the smaller the relative richness of the capillary network containing the gelatin.



# EXPERIMENTS ON THE DEVELOPMENT OF BLOOD VESSELS IN THE AREA PELLUCIDA AND EMBRYONIC BODY OF THE CHICK

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THIRTEEN FIGURES

Those who have studied the early development of blood vessels in the amniote embryo agree that the formation of blood islands in the area opaca of the blastoderm represents the first stage in vascularization. The first blood islands appear about the time the primitive streak reaches the height of its development, in the peripheral part of the mesoderm that lies caudal to the primitive streak. Then, as the 'head process' (primitive axis) develops, they extend forward in the peripheral part of the mesoderm that lies lateral to the primitive streak and 'head process.' The blood islands thus outline a crescent-shaped area in the blastoderm—the area vasculosa. Subsequently the cells of these blood islands differentiate into primitive blood cells and the endothelium of the primitive blood vessels.

While it is clear that the first blood vessels, namely, those in the area opaca of the blastoderm, arise in situ, the manner in which the region within the concavity of the crescentic area vasculosa, constituting the area pellucida and embryonic body, becomes vascularized has been the subject of much controversy. The views expressed by different investigators can be grouped under two heads, as follows: (1) The vessels that appear in the area pellucida and embryo subsequent to vessel formation in the area opaca are derived from sprouts which grow in

from the already formed vessels or islands. (2) They arise in situ in a manner essentially like that in which the vessels in the area opaca are formed.

The view that vascularization of the area pellucida and embryonic body is the result of ingrowth from the islands or vessels already present in the area opaca has associated with it primarily the name of His (1). This view was acquiesced in by Türistig (2), Vialleton (3), and others, and in recent years Evans (4), Minot (5), and Bremer (6) also have asserted their belief in ingrowth of vessels from the area opaca.

The second view, namely, that the blood vessels of the area pellucida and embryonic body arise in situ and are not the result of ingrowth from antecedent vessels or islands in the area opaca, is most strongly advocated by Rückert and Mollier (7). The recent work of McWhorter and Whipple (8) on the growth of chick blastoderm in vitro also supports this view.

The writers have here attempted to derive from experimentation on the living blastoderm of the chick some evidence bearing on the question of vascularization of the area pellucida and embryonic body. It seemed reasonable to assume that, if the lateral half of the entire area opaca was cut off or in some way removed from the blastoderm at a time when no blood vessels or angioblast (in the sense of Minot and Bremer) had appeared in the area pellucida or embryonic body, and the blastoderm was then allowed to proceed in development, it would be possible to test the validity of the view that blood vessels in the area pellucida and embryo arise in situ and not as ingrowths or sprouts from the antecedent angioblast, blood islands or vessels in the area opaca.

The first step in our study was, therefore, to determine the time to operate on blastoderms in order to exclude the possibility of ingrowth of angioblast, if such there might be, from the area opaca. This was done by examining serial transverse sections of blastoderms from a stage prior to the appearance of the primitive streak up to a stage in which one or two pairs of somites were present. This examination showed that up to the stage in which the 'head process' was clearly visible on surface

view there were no cells between mesoderm and entoderm in the area pellucida or embryonic body.

In view of this fact, therefore, we sought to remove the lateral half of the area opaca at a stage not later than the complete formation of the primitive streak, thus allowing a considerable interval between the time of operation and the normal time of appearance of blood vessel anlagen in the area pellucida and embryonic body. In brief, our aim was to prevent any possible ingrowth of 'angioblast' from the lateral portion of the area opaca of one side. This accomplished, it would follow that any vessels which appeared subsequent to operation, in the remnant of the area pellucida or in the same side of the embryonic body, must have arisen in situ.

#### TECHNIQUE

Eggs of the common fowl were incubated for twenty hours at a temperature of 38°C. On removal from the incubator a sufficient quantity of shell was removed to expose the whole upper surface of the yolk. With the aid of the binocular microscope, the stage to which the blastoderm had developed was ascertained and if it was found not to have progressed beyond the primitive streak stage an incision was made with a pair of very fine scissors parallel to its long axis. This incision was made to extend well beyond the periphery of the blastoderm and as close to the primitive streak as possible, without injuring it. In a number of embryos, in addition to the incision already mentioned, another cut was made at an angle of 45 degrees with the first, beginning at the caudal end of the primitive streak and extending diagonally across behind it, as indicated in figure 1. This second cut was made in order to prevent possible vasofactive cells on this side from proceeding forward along the primitive streak. As soon as the incision has been made the wound gapes widely, thus very effectively separating the two portions of the blastoderm. At the same time other blastoderms of the same stage were removed from the yolk, fixed, sectioned serially and stained. A section of one of these control specimens is represented in figure 2.



The blastoderm being ready for further incubation, the eggs were carefully placed in small specimen dishes, with loosely fitting glass covers, and surrounded by cotton. The cotton was saturated with some solution, usually Ringer's, the object of this being to keep the exposed surface of the yolk moist and thus to prevent destruction of the blastoderm by drying. These dishes were placed in the incubator and the blastoderms allowed to incubate from sixteen to seventy-two hours longer at a temperature of 38°C. In the majority of cases the incubation period was from twenty to twenty-two hours.

Approximately fifty blastoderms were operated upon. Subsequent development in the incubator took place with remarkably few losses. Not more than 10 per cent of the blastoderms died and these apparently from excessive dryness in the dishes and not from the injury inflicted.

On completion of a given period of incubation, the egg was removed from the incubator and a circular incision was made in the blastoderm beyond the sinus terminalis. With a spatula the embryo, with its adherent membranes, was lifted from its position on the yolk and immersed in Ringer's solution at incubation temperature. Yolk granules and vitelline membrane were removed by gently squirting, through a small pipette, jets of the solution against the embryo. The embryo was then transferred to a glass slide and fixed in either Zenker's or Mann's fluid.

All specimens were photographed in dorsal view before embedding, for purposes of comparison in the study of sections and in making reconstructions. The specimens were then embedded in paraffin and cut in serial sections of from 5 to 6 microns in thickness. The sections were mounted on slides in the usual manner and then stained by one of the following methods: Heidenhain's or Weigert's hematoxylin, Giemsa's eosin and azur II, or Dominici's toluidin blue, eosin and orange G.

## GROSS EXAMINATION

After removal from the yolk, some of the blastoderms were examined in the warm Ringer's solution under the binocular microscope. Apart from a slight retardation, development of the embryonic body had gone on in many cases in an apparently normal manner up to from twenty to twenty-four hours after operation, and the growth of the extra-embryonic area on the uninjured side had followed the usual course. In other cases, in which the incision had been made too close to the primitive streak, the embryo was abnormal in that it lacked a neural fold and mesodermic somites in part on the injured side. The heart could always be clearly seen, and under sufficient magnification the blood cells could be seen circulating in the vessels that had already joined with the heart.

Embryos that were allowed to develop for more than twenty-four hours after operation usually became abnormal in contour, or even monstrous, although the extra-embryonic area with its blood vessels continued to develop fairly regularly. The heart continued to beat and the circulation went on as usual up to seventy-two hours after operation, the longest period we allowed any of the blastoderms to develop. In the living specimens it was difficult, on account of the thickness of the tissues, to determine the conditions between the embryonic body and the line of incision. Blood islands were usually visible and, in later stages, faint outlines of blood vessels.

In figure 3, taken from a photograph of a fresh specimen twenty-four hours after operation, the embryonic body and the mesodermic somites (9), of which ten pairs are present, are plainly visible. This shows especially well the advance in development during the first day after operation. Compare with figure 1, taken from a blastoderm at the stage of a fully developed primitive streak (1)—the stage at which we operated. On the uninjured side in figure 3 the blood islands (10) show distinctly in the peripheral part of the area vasculosa (11). While the vessels in the area pellucida (through which blood was passing freely) do not

show, the heart (12) is seen at the right of the head region. On the injured side, between the embryonic body and the line of incision, the darker shading represents blood islands (10a).

Figure 4, taken from a specimen in the clearing fluid (cedar oil), shows an embryo which had been allowed to develop forty-eight hours after operation and had grown for that period in a fairly normal manner. The embryo itself is well outlined and exhibits the various parts of the developing nervous system as well as the somites (9), of which 15 pairs are present. The extra-embryonic area, with its clearly marked blood vessels (11), is relatively smaller than in most of the other specimens. The heart (12) is very distinct, and, as shown by subsequent study of sections, is unilateral; that is, formed from the anlage of the right side, the left anlage being wholly lacking. On the injured side the darker areas (11a) between the line of somites and the line of incision represent large aggregations of developing blood cells within developing vessels.

#### MICROSCOPIC STUDY AND RECONSTRUCTIONS

The reconstructions were made by the graphic method from drawings of serial transverse sections. The drawings were made with the aid of the Edinger projection apparatus, and each section was then carefully studied under high magnification and the corresponding drawing thus verified before being plotted.

In figure 5 is reproduced a partial reconstruction of the vessels and masses of developing blood cells in an embryo which had been allowed to develop twenty-four hours after removal of the left half of the area opaca. All the vessels and blood cells on the injured side are represented. On the uninjured side only the aorta and its branches are shown, the plexus of vessels in the extra-embryonic area being omitted.

On the right (uninjured) side the dorsal aorta (14) follows the usual course. Near its caudal end it sends branches (15) to the extra-embryonic area. These branches constitute the part of the original plexus out of which the vitelline artery, as well as the aorta, is formed. Two small branches farther cephalad



also represent remnants of this plexus. So far as conditions in general are concerned, therefore, the aorta and its branches on the uninjured side developed normally. At its extreme caudal end the aorta becomes continuous with a small solid cord of cells which resemble the cells of a blood island, the endothelium merging with the superficial elements of the mass.

On the injured side, the dorsal aorta (*14a*) is much smaller than its neighbor of the uninjured side. Through the major part of its course it is a perfectly distinct vessel and possesses a complete endothelial wall (fig. 6, *14a*). It contains but few free blood cells. The caudal third is composed in large part of a solid cellular cord (figs. 5 and 7, *14b*). In this cord are four distinct and separate spaces (figs. 5 and 8, *14c*), which represent the lumen of the vessel. The solid portion is composed of cells which, considering their structure and their reaction to dyes, are identical with the cells in the blood islands of the normal area opaca. It is, therefore, justifiable to conclude that this cord is comparable to a blood island. This seems all the more justifiable in view of the fact that the cells surrounding the spaces in the cord are flattened, unquestionably representing endothelium (figs. 5 and 8, *14c*), and merge with the superficial cells of the solid masses.

From these conditions we may conclude that the caudal third of the aorta on the injured side is in process of formation from a structure identical with a blood island in the same manner as a vessel is formed from a blood island in the area opaca.

The somites and intermediate cell masses on the injured side in the embryo apparently developed in a normal manner (fig. 7, 9, *15*). The coelom (*16*), while somewhat irregular, is well formed. The incised edges of the ectoderm and entoderm have fused, the cut thus being healed. The fusion of these two layers is not only interesting in itself but also shows the slight degree of injury inflicted in cutting the blastoderm (fig. 7, *x*).

Just mesial to the line of incision, in the splanchnic mesoderm, a large blood island extends in an unbroken line from about the middle of the embryo to its caudal end (fig. 5, *10a*). Cephalad it is continuous with a small but distinct vessel which opens into

the caudal end of the heart and is therefore probably analogous to the vitelline vein. The blood island is in every respect similar to the ordinary blood island of the area opaca. The cells are rounded, closely packed together, and possess strongly basophilic cytoplasm. In a few places the superficial cells are somewhat flattened, thus indicating the formation of endothelium. Near the cephalic end of the island are three distinct and separate spaces with perfect endothelial walls. From a point near the caudal end of the island a much smaller cord of cells extends obliquely forward toward the aorta. In this cord are several spaces walled by flattened cells. Between this extensive blood island and the aorta no direct connection could be discerned even under high magnification. Near the caudal end of the large blood island (10a) are several small isolated islands (10b) and one isolated space (10c) lined with endothelium.

In figure 9 is reproduced a reconstruction of the vessels in an embryo which has been allowed to develop for forty-eight hours after removal of the left half of the area opaca. In this specimen the germ layers did not close in ventrally to form a body wall and gut (see fig. 4; cf. figs. 10, 11 and 12), probably as a result of the proximity of the incision to the axial line.

On the uninjured side the extra-embryonic vascular area developed normally, excepting its less than normal size. The dorsal aorta (14) also developed normally, and near its caudal end becomes continuous with the general plexus of vessels in the extra-embryonic area. The vessels of the cephalic portion of the extra-embryonic plexus converge to form a main trunk, which opens into the caudal end of the heart (12) and represents the vitelline vein.

The heart itself (12) is unilateral, that is, not fused with a corresponding anlage of the opposite side, and is situated in the splanchnopleure some distance from the medial line (fig. 10, 12). This is about the position the lateral anlage of the heart occupies in earlier stages in normal embryos, before it meets its fellow of the opposite side. Examination of this embryo in warm Ringer's solution showed the heart to be beating regularly. The direction of circulation is indicated by the arrows in figure 9.

On the uninjured side the heart is continuous cephalad with the ventral aorta (fig. 9, 19) which lies close to the entoderm within the embryo proper (fig. 10, 19). In its course the ventral aorta gives off three aortic arches (fig. 9, 20, 21, 22) which open into a vessel lying quite close to the ventro-lateral wall of the neural tube. This vessel, the cephalic portion of the dorsal aorta (figs. 9 and 10, 23), becomes continuous with the dorsal aorta of the trunk (fig. 9, 14). The dorsal aorta at its cephalic end also communicates with the lateral vein of the head (fig. 9, 24) which lies along the dorso-lateral aspect of the neural tube (fig. 10, 24).

The lateral vein of the head (figs. 9 and 10, 24) extends from the fore-brain region, where it receives a branch from the opposite side (fig. 9, 24a), along the dorso-lateral aspect of the neural tube to the level of the caudal end of the heart (12). At this point it expands laterally in the somatopleure into a rather extensive plexus which lies in the region of the amnio-embryonic angle. This plexus undoubtedly represents the beginning of the umbilical vein (figs. 9 and 11, 25). At the dotted circles in figure 9 it opens into the caudal end of the heart through two small channels which pass around the coelomic angle. In line with the lateral vein of the head, but farther forward in the forebrain region, is a large sinus which does not communicate with any other vessel (fig. 9, 24b).

The dorsal aorta (fig. 9, 14) along the cephalic part of its course gives off a number of dorsal branches. Some of these apparently terminate in the mesenchymal intercellular spaces along the neural tube. Others pass to the lateral side of the mesodermic somites and terminate in intercellular spaces. Still others passing lateral to the somites, expand at their distal ends, especially in the longitudinal direction, and in a few cases join one another to form a longitudinal channel. This longitudinal channel, together with the expanded ends of the other laterally directed branches of the aorta apparently represent the beginning of the cardinal vein (figs. 9 and 12, 26).

On the injured side of this embryo the conditions in some respects are notably similar to those on the uninjured side; in



other respects there are certain differences which can be accounted for only on the basis of the different circumstances due to the injury. The dorsal aorta (fig. 9, 14a) in the major part of the course is a well-formed vessel occupying the same relative position as, but smaller than, its fellow of the opposite side (14); (see also figs. 11, 12 and 13, 14a). In the caudal region it expands laterally into a plexus of vessels in the remaining portion of the extra-embryonic splanchnopleure (fig. 9, 11a). Cephalad it is continued in a dilated vessel (23a) which is probably the cephalic aorta (23). This is connected with a more dorsally situated sinus (figs. 9 and 10, 24c), which represents the lateral vein of the head (24). The two dilated vessels together form a relatively enormous sinus which extends along the lateral aspect of the brain almost to the extreme cephalic end of the embryo. The aorta of this side (14a) is fused with that of the opposite side (14) in three places, as indicated in figure 9; (see also fig. 11, 14, 14a).

The lateral vein of the head on the injured side (fig. 9, 24c) is continued caudad by a series of vessels situated lateral to the mesodermic somites and probably equivalent to the cardinal vein of the opposite side (figs. 9, 11 and 12, 26a). At two levels, as represented in figure 9, this series (26a) communicates with the aorta (14a). While for the most part these vessels are lined with a distinct endothelium, near or at their ends they apparently open freely into the surrounding mesenchymal intercellular spaces, the endothelium merging with the undifferentiated mesenchymal cells.

A number of isolated lacunae (27), in part lined with endothelium and in part opening into the surrounding tissue spaces, are situated along the lateral aspect of the neural tube. Several other lacunae of a similar character, one especially large (28), are situated more peripherally.

The vessels and lacunae on the injured side thus far described are almost destitute of blood cells, a few being present in the large sinus of the head region. The plexus of developing vessels (11a) in the extra-embryonic splanchnopleure contains, however,

a great number of blood cells, as shown in figure 13 (11a). In some localities the endothelial wall of the vessel is completely formed, the blood cells being contained within the lumen (fig. 13, 11a). In other localities the conditions resemble those of a blood island in which the superficial cells are just in process of flattening. In figure 9 (29) there is represented a blood island that in section consists merely of an accumulation of round cells with basophilic cytoplasm. Typical blood island structure is also present in the caudal portion of the extra-embryonic plexus (fig. 9, 11a).

In this embryo, as in the 24-hour embryo, the cut edges of the ectoderm and entoderm have fused (figs. 10, 11, 12 and 13). The coelom on the injured side has developed in the form of irregular communicating spaces, which appear distended and suggest an accumulation of fluid here in a region not as freely drained as on the opposite side, where there was uninterrupted circulation in the blood vessels (fig. 7, 16).

These two embryos, which we have described in detail, have been selected as typical of the whole of our series of experiments, in all of which we found active vasculogenesis in the area pellucida and embryonic body mesial to our incision. The gap formed as a result of operation remained permanently open, and definitely precludes the possibility of continuous ingrowth of angioblast cords such as Bremer has described in the rabbit. The conditions described show that the removal of the entire lateral half of the area opaca at a stage prior to the appearance of recognizable vasofactive cells or vessel rudiments in the area pellucida does not prevent subsequent development of blood cells and blood vessels in the remaining portion of the area pellucida or in the embryonic body on the same side.

On the other hand, it has been found that after this injury blood islands appear in the remaining portion of the extra-embryonic mesoderm and even in the embryonic body; that from these blood islands both endothelium and blood cells are evolved in the usual manner; and that, in addition, blood vessels destitute of blood cells develop in both localities.

We conclude, therefore, that the same processes are at work in the formation of endothelium in the embryo and in its membranes, and that the blood vessels of the embryo do not result from the ingrowth of a formed endothelium, but arise in situ from an indifferent mesenchyme.

#### ADDENDUM

While this article was in press there came to our attention the work of H. Hahn (*Experimentelle Studien über die Entstehung des Blutes und der ersten Gefäße beim Hühnchen. I. Teil. Intraembryonale Gefäße. Arch. f. Ent.-Mech. d. Organismen. 27 Bd. 1909*). Hahn's brilliant investigation, it seems to us, has not received due credit, if we may judge from the fact that the hematological literature which has appeared since the publication of his work has almost entirely disregarded his conclusions and the evidence on which they are based. It is an agreeable duty for us here to acknowledge the full value of Hahn's prior investigations and results which were obtained through a long series of painstaking experiments and with which our conclusions are in complete harmony.

It may be noted, however, that three points of difference obtain in the methods employed in the two cases. (1) Hahn used the electric cautery to destroy the caudal portion of a lateral half of the blastoderm at the early primitive streak stage. We cut off with scissors the entire lateral portion of the blastoderm, making the incision as close to the primitive streak as possible. This procedure reduced to a minimum the amount of damage, thus lessening interference with subsequent development of the germ layers. The probability is that the incision more effectively removes the lateral portion of the blastoderm, with less general injury than burning; and thus the conclusion that the vessels which subsequently develop within the embryo arise in loco is rendered even more certain. (2) By Hahn's method the site of origin of the heart was probably not destroyed. By the cutting method, if the incision is made close enough to the primitive streak, the site of heart development is removed. In many of our specimens no heart rudiment appeared on the injured side; yet the intraembryonic vessels developed. The view, therefore, that these vessels are derived from the heart rudiment (Rabl) is no longer tenable. (3) While Hahn made in toto graphic reconstructions of some of his blastoderms, he did not reconstruct the intraembryonic vessels, although figuring them beautifully in sections. Graphic reconstructions were made of the vessels in our embryos in a number of cases, from which a more comprehensive view of the vascular system can be obtained. These differences in method have led, however, not to different results, but to conclusions which are wholly in accord and which seem to leave but little doubt as to the validity of the view that intraembryonic vessels are not derived from extrinsic vascular anlagen but arise in situ.

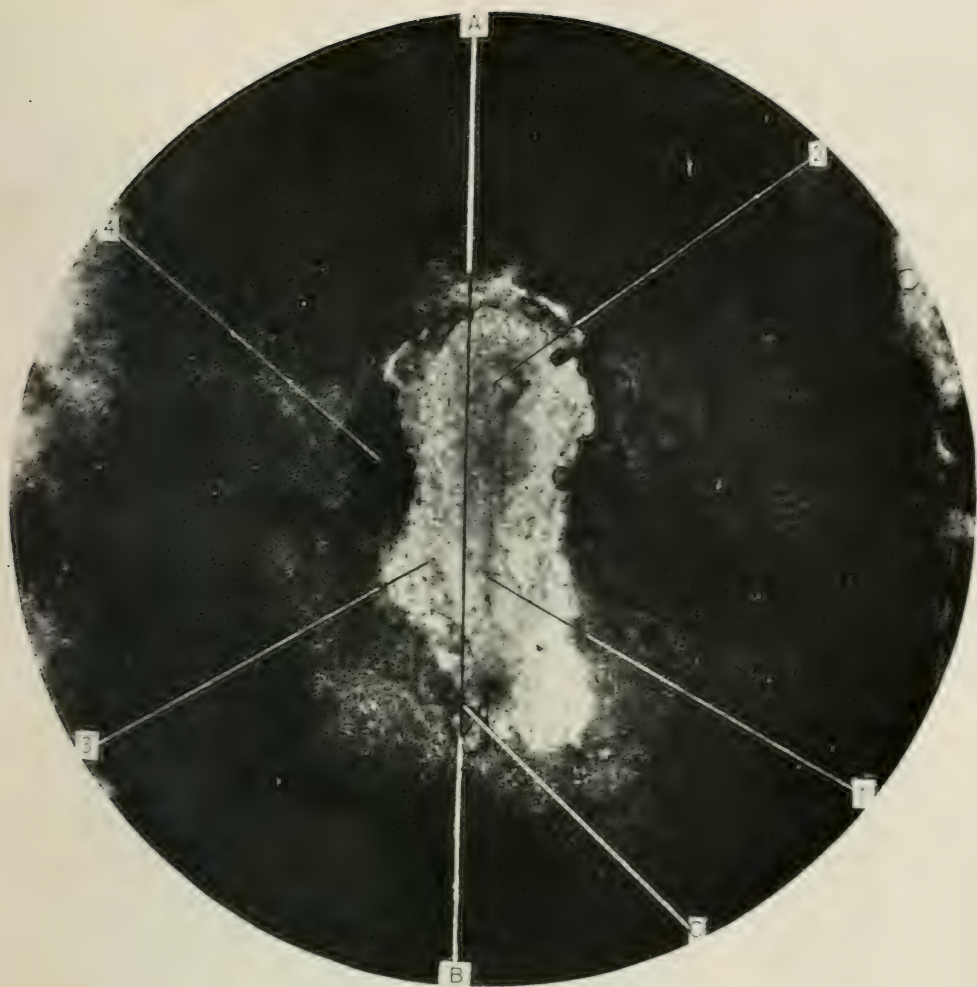


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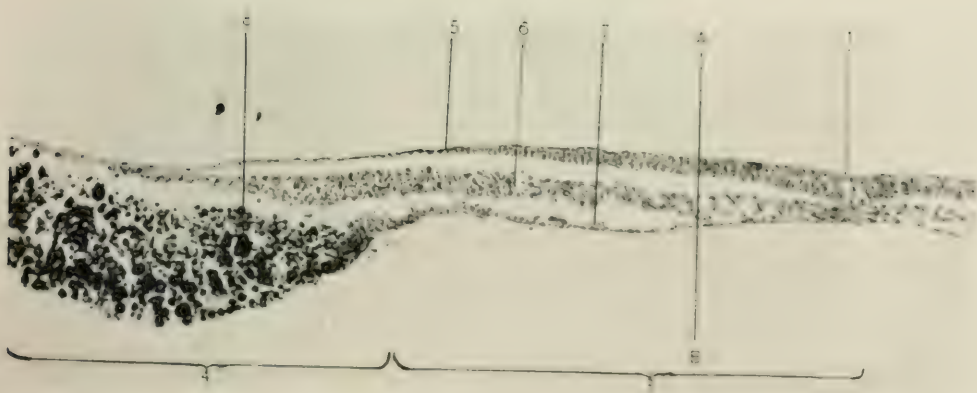
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Fig. 1 Dorsal view of a chick blastoderm of twenty hours incubation, showing the fully formed primitive streak (1), primitive node (2), area pellucida (3), and area opaca (4). Photomicrograph. (Columbia Embryological Collection, series No. 602.) The line *A-B* indicates the direction of the main incision made to remove the area opaca on one side. The line projected to *C* indicates the second incision made in some cases.

Fig. 2 From a transverse section through the primitive streak (1), area pellucida (3), and area opaca (4), of a chick blastoderm of twenty hours incubation (stage of fully formed primitive streak). Photomicrograph. (Columbia Embryological Collection, series No. 611, slide VI, section 40.) 5, Ectoderm; 6, mesoderm; 7, entoderm; 8, germ wall. The line *A-B* indicates the main incision made to remove the area opaca (4).



1



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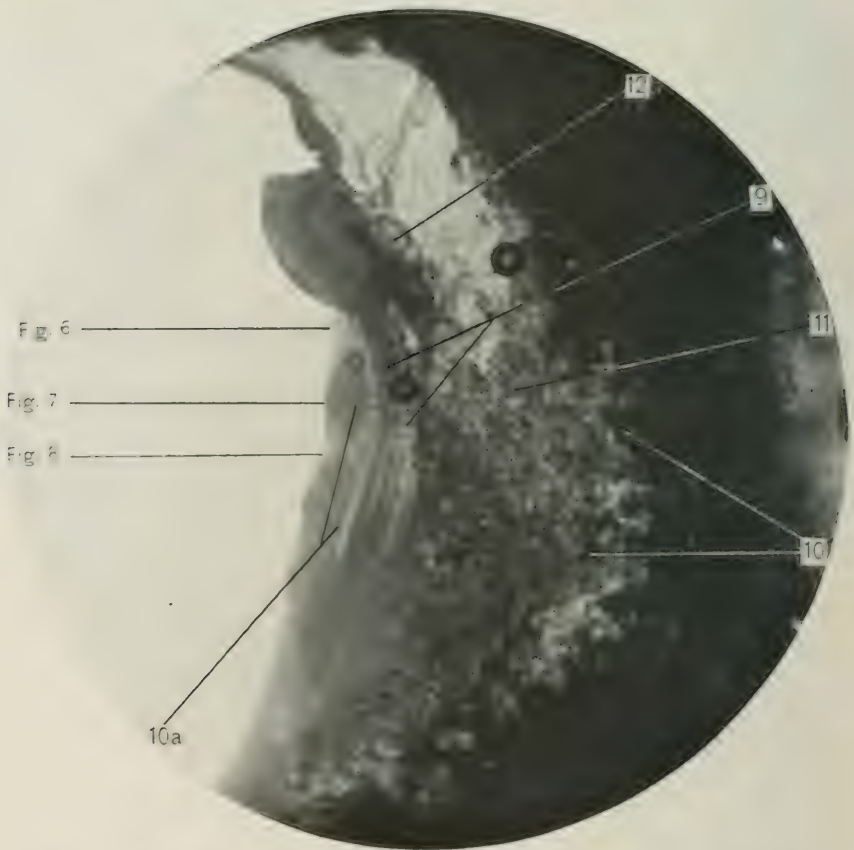


Fig. 3 Dorsal view of a chick blastoderm which had been allowed to develop for twenty-four hours after removal of the left half of the area opaca in the manner indicated in figures 1 and 2. Photomicrograph of fresh specimen. (Columbia Embryological Collection, series No. 600.) 9, Mesodermic somites; 10, blood islands in splanchnic mesoderm on uninjured side; 10a, blood islands in splanchnic mesoderm on injured side; 11, area vasculosa on uninjured side; 12, heart. Levels at which sections shown in figures 6, 7 and 8 were taken are shown at left of figure.

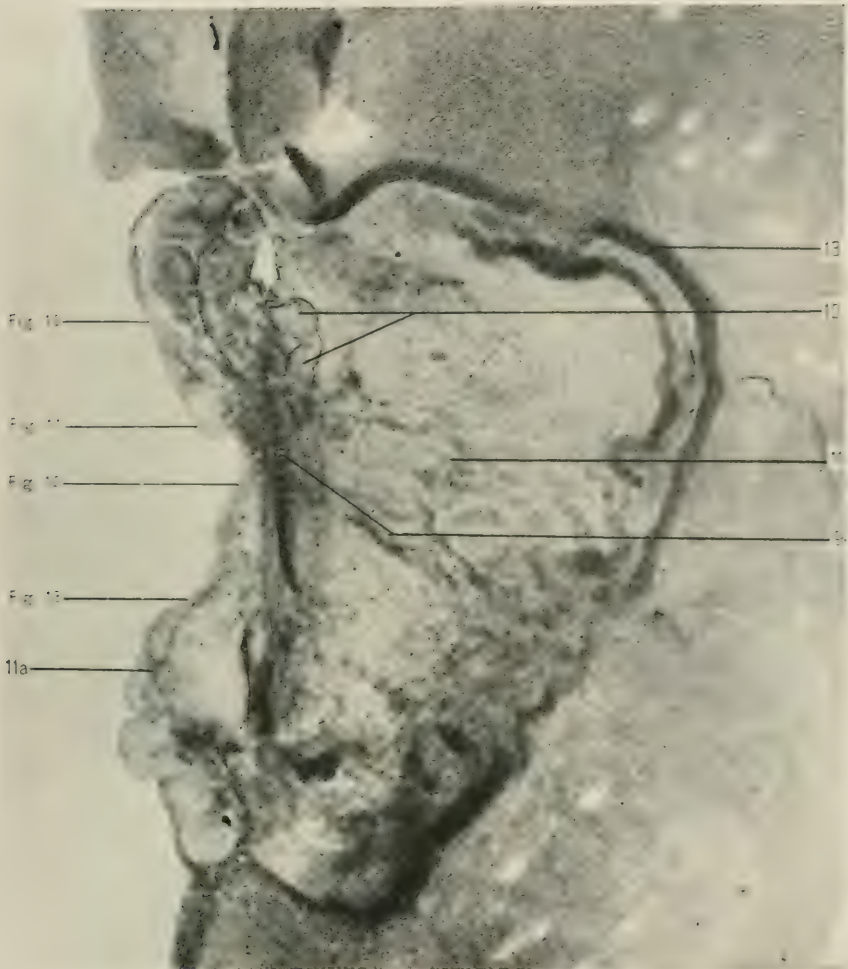
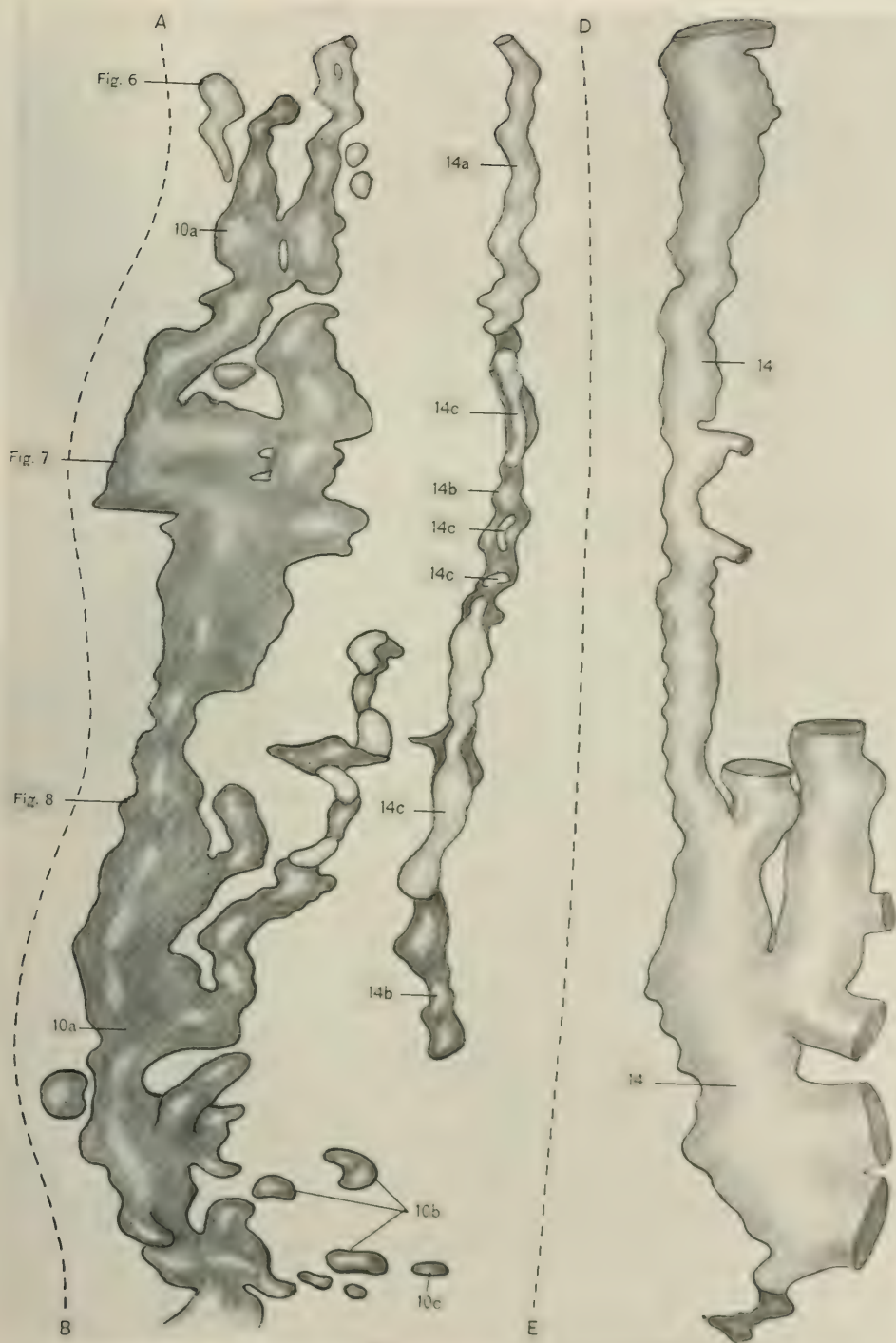


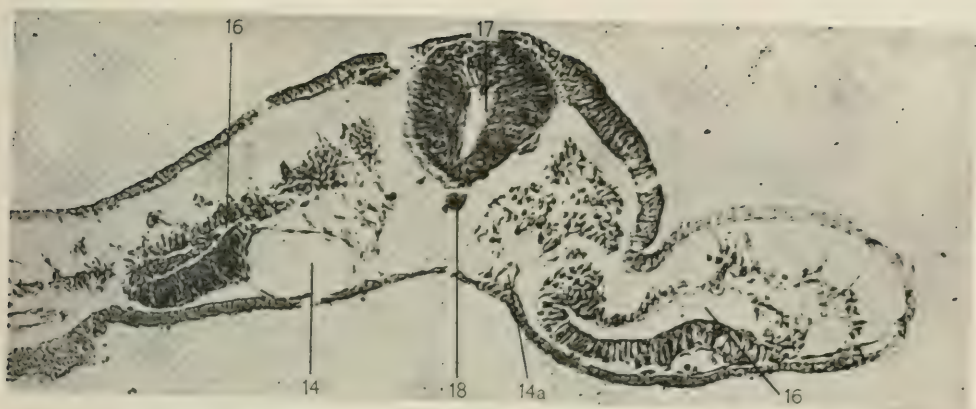
Fig. 4 Dorsal view of a chick blastoderm which had been allowed to develop for forty-eight hours after removal of the left half of the area opaca in the manner indicated in figures 1 and 2. Photomicrograph. (Columbia Embryological Collection, series No. 613.) 9, Mesodermic somites; 11, area vasculosa on uninjured side; 11a, developing blood vessels in splanchnic mesoderm on injured side; 12, heart; 13, sinus terminalis. Levels at which sections shown in figures 10, 11, 12 and 13 were taken are shown at the left.

Fig. 5 From a partial reconstruction of the blood vessels and developing blood cells in an embryo which had been allowed to develop for twenty-four hours after operation. Same embryo as in figure 3. Dorsal view. *10a*, Large blood island in splanchnic mesoderm on injured side (cf. figs. 6, 7 and 8, *10a*); *10b*, small isolated blood islands; *10c*, small isolated lacuna; *14*, aorta on uninjured side; *14a*, *14b*, *14c*, aorta on injured side. Dotted line *A-B* indicates line of cut edge of germ layers (cf. figs. 6, 7 and 8); dotted line *D-E* lies in mesial sagittal plane of embryo. Levels at which sections shown in figures 6, 7 and 8 were taken are indicated at left of figure.





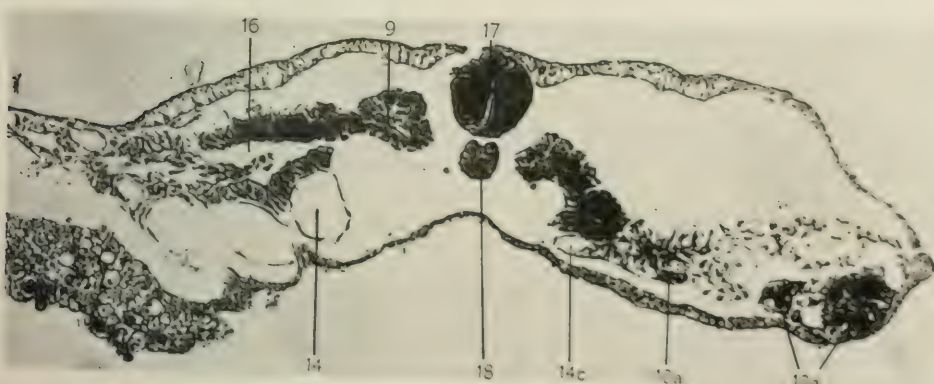
Figs. 6, 7 and 8 From transverse sections of same embryo as in figures 3 and 5 in which the levels of these sections are indicated at the left. Photomicrographs, slide XI, section 12 and slide XII, sections 7 and 35, respectively. 9, Mesodermic somites; 10a, blood island in splanchnic mesoderm on injured side; 14, aorta on uninjured side; 14a, 14b, 14c, aorta on injured side (see fig. 5); 15, intermediate cell mass; 16, coelom; 17, neural tube; 18, notochord. At the point X in figure 7 the incised edges of the ectoderm and entoderm are shown to be fused.



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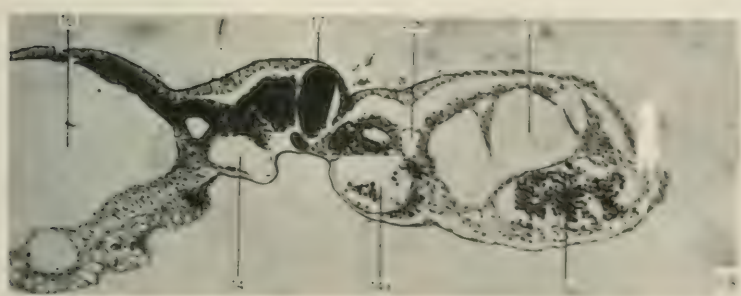
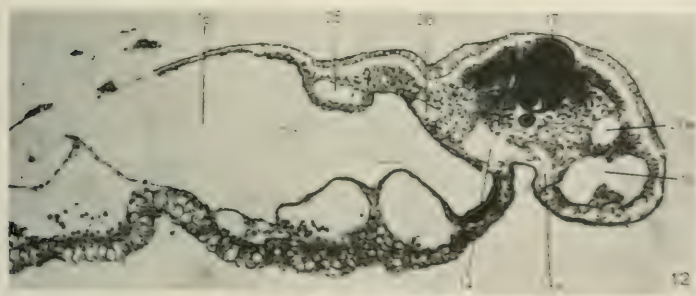
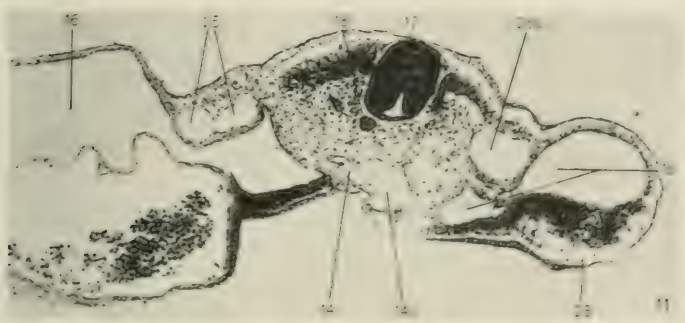
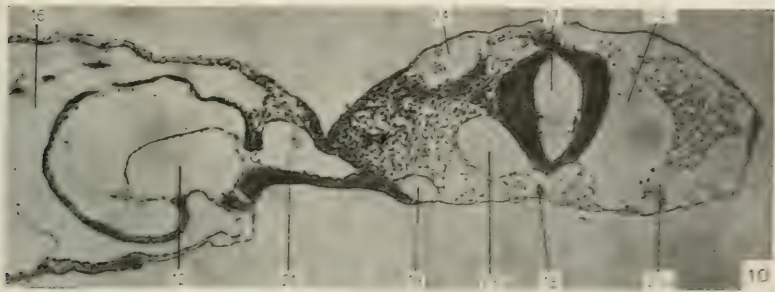


Fig. 9 From a reconstruction of the blood vessels in an embryo which had been allowed to develop for forty-eight hours after operation. Same embryo as in figure 4. Dorsal view. 11, Vessels in extra-embryonic splanchnic mesoderm (area vasculosa) on uninjured side; 11*a*, developing blood vessels in splanchnic mesoderm on injured side; 12, heart (unilateral); 14, aorta on uninjured side; 14*a*, aorta on injured side; 19, ventral aortic root; 10, 21, 22, aortic arches; 23, dorsal aortic root on uninjured side; 23*a*, dorsal aortic root (?) on injured side; 24, lateral vein of head on uninjured side; 24*a*, branch of same from opposite side; 24*b*, isolated portion of same; 24*c*, lateral vein of head on injured side; 25, umbilical plexus; 26, cardinal vein; 26*a*, cardinal vein on injured side; 27, small lacunae lateral to neural tube; 28, large lacunae in splanchnic mesoderm; 29, large typical blood island in somatic mesoderm. Dotted line *A-B* indicates line of cut edge of germ layers (cf. figs. 10, 11, 12 and 13). Levels at which sections shown in figures 10, 11, 12 and 13 were taken are shown at the left.



Fig. 10, 11, 12 and 13 From transverse sections of same embryo as in figures 4 and 9 in which the levels of these sections are indicated at the left. Photomicrographs. Slide V, section 54, slide VI, section 53, slide VII, section 22, slide VIII, section 38, respectively. 11a, Blood vessels in splanchnic mesoderm on injured side; 12, heart; 14, aorta on uninjured side; 14a, aorta on injured side (fused with 14, in fig. 11); 16, coelom; 17, neural tube (lacking left half in fig. 13); 18, notochord; 19, ventral aortic root; 21a aortic arch; 23, dorsal aortic root on uninjured side; 23a, dorsal aortic root on injured side; 24, lateral vein of head on uninjured side; 24c, lateral vein of head on injured side; 25, umbilical plexus; 26, cardinal vein on uninjured side; 26a, cardinal vein on injured side; 28, portion of large lacuna represented in figure 9.





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# THE DISTRIBUTION OF NERVES TO THE ARTERIES OF THE ARM

J. G. KRAMER

## WITH A DISCUSSION OF THE CLINICAL VALUE OF RESULTS

T. WINGATE TODD

*From the Anatomical Laboratory, Western Reserve University, Cleveland*

FIVE FIGURES

### I. INTRODUCTION

Our knowledge concerning the vascular nerves of the body is exceedingly scanty. The standard textbooks contain only scattered and incomplete references to the nerve-supply of bloodvessels. The few isolated papers dealing with the subject are inadequate and do not give clear accounts which can be utilized in clinical practice. For these reasons Mr. Wingate Todd has advised the reinvestigation of the vascular nerves in homo and at his suggestion I have undertaken the dissection of the nerve-supply to the arteries in the arm. The importance of the work was first impressed on Mr. Todd by Prof. Elliot Smith of Manchester, whose description of a vascular nerve to the axillary artery formed the starting point of the present research and to which reference will be made later.

The following is a brief abstract of the statements made in various standard textbooks regarding the nerve-supply of the bloodvessels of the arm:

Hamann, writing in Piersol's Anatomy (1), mentions (a) the supply of fibers to the subclavian artery and its branches from the ansa subclavia (p. 1362), the distribution of filaments (b) to the volar interosseous artery from the corresponding nerve (p. 1301) and (c) to the ulnar artery in the forearm from the palmar cutaneous branch of the ulnar nerve (p. 1305).

Paterson in Cunningham's Textbook, (2) refers to the branch supplied by the musculo-cutaneous nerve to the brachial artery (p. 705) and mentions the first and third groups of filaments in Hamann's description.

In Quain's textbook Symington (3) describes, in addition to those mentioned by Hamann, a branch from the musculo-cutaneous nerve to the brachial artery (p. 75). The irregularity in precise origin of this nerve is indicated by the fact that it was found by Testut coming from the branch to the *M. brachialis*.

The textbooks known as Morris' (4) and Gray's (5) Anatomies do not give any further information regarding the vascular nerves of the arm.

Rauber's textbook also was consulted without obtaining further information (6).

Soulié's description in Poirier's treatise (7) mentions the distribution of filaments (a) to the brachial artery from the median nerve (p. 907), (b) to the volar ulnar recurrent artery from the volar interosseous nerve near its origin (p. 908), (c) to the ulnar artery in the forearm from the palmer cutaneous branch of the ulnar nerve (p. 919), and (d) to the radial artery and vein from the volar terminal branch of the musculo-cutaneous nerve. He also describes the direct supply of filaments to the subelavian artery and its branches from the inferior cervical ganglion (p. 1094).

From the preceding abstract it is clear that the references scattered through the various descriptions are by no means connected or complete.

While one expects variations to be found in the origin of supply from the sympathetic chain to the subelavian artery, one would like to know whether the same irregularity is to be encountered in the nerve-supply to the other arteries of the arm.

The fact that Elliot Smith describes a nerve twig, arising from the lateral anterior thoracic nerve, to the axillary artery in a case exhibiting multiple anomalies of nerves (8), illustrates the contention that variation is to be noticed among vascular nerves as among nerves to muscle and skin. It is, of course, plain from the outset that the view that the sympathetic vascular nerves pass along the main blood channels to their distribution on the peripheral vessels, is quite inaccurate. This view and the evidence for its refutation have already been discussed by Mr. Todd (9), hence I may, without further delay, set down my own observations on the vascular nerves of the arm.



## II. PERSONAL OBSERVATIONS

The dissections were made on six upper limbs. Five of these came from cadavera in the Anatomical Department. One hand and forearm was obtained from Dr. Hamann's operating theatre. This last was dissected within a day or so of amputation in order to confirm the observations made on the cadavera. It may be well to remark at this stage that the dissection of nerves to bloodvessels is much easier on freshly obtained material and also that the embalming of the subject with more than a small percentage of formalin renders difficult the identification of these small nerves from bundles of fascia or connective tissue. The nerves mentioned in this paper were readily identifiable by the naked eye, all doubtful filaments being rejected. As the work progressed it became plain that the recording of every individual nerve filament is impossible and also that there is considerable variation in the exact site of origin of the filament from the nerve and in the situation where it reaches the artery in different arms. That is, the sympathetic fibers to the vessels of the limb are as erratic in their precise origin and distribution as are those in the trunk.

The subclavian and proximal part of the axillary arteries receive a nerve-supply directly from the sympathetic chain, between or including the middle and inferior cervical ganglia. In the specimens I have examined, the nerve was moderate in size and reached the artery on its inferior aspect, proximal to the passage of the vessel over the first rib. It accompanied the artery in the interval between the M. scalenus anterior and the bone (fig. 1). The portion of the subclavian artery immediately adjacent to its origin was supplied by twigs from the ansa subclavia. I did not find further branches of supply to the axillary artery from the lateral anterior cutaneous nerve (see Elliot Smith '95). The brachial artery was supplied by a varying number of twigs from the musculo-cutaneous nerve. In one case, I met with an example of high division of the brachial artery, which is depicted in figure 2. The nerve-supply in the illustration differs in no essential point from that of normal brachial arteries.

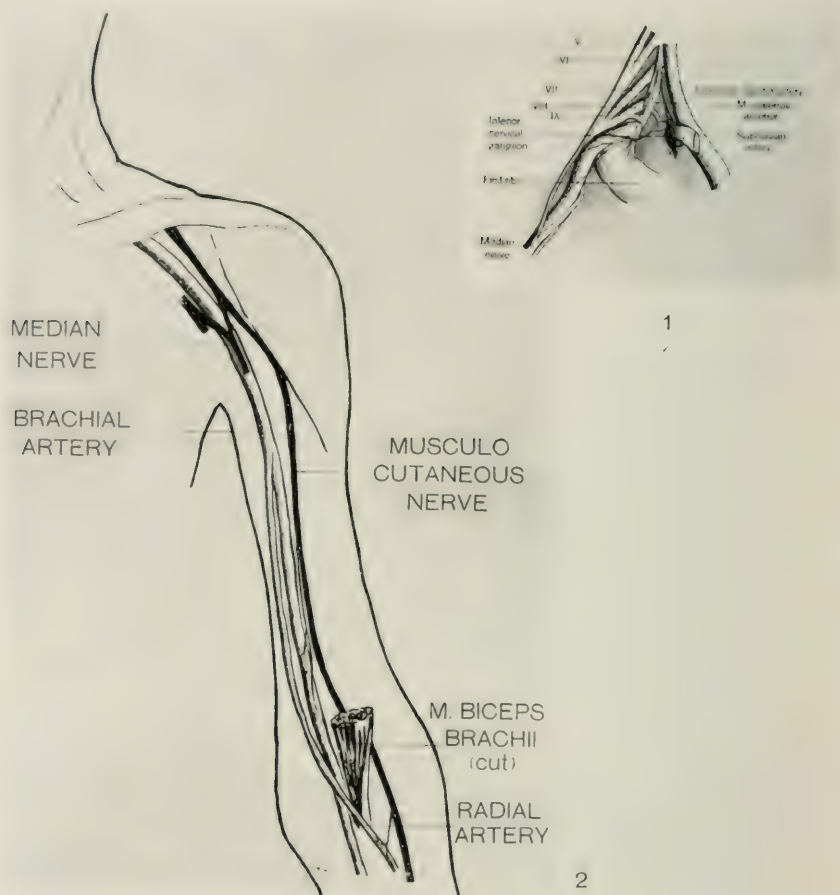


Fig. 1 Right subclavian artery, to show nerve-supply. In this case the comparatively large filament arose by two roots from the inferior cervical ganglion and the sympathetic chain immediately above.

Fig. 2 Diagram of nerve-supply to the brachial artery. This example showed high division of the vessel, but illustrates as well as a normal specimen the supply from the musculo-cutaneous nerve. Note that the same nerve supplies an additional branch to the proximal part of the radial artery.

Each figure represents an individual instance, but may be taken as a fairly typical illustration of the general plan of vascular nerves. The illustrations are reduced to one-fourth normal size.

The radial artery, as can be seen from figure 3 received a nerve-supply from the superficial ramus of the radial nerve. The dorsal carpal arch and its branches also received twigs from this nerve (fig. 5).

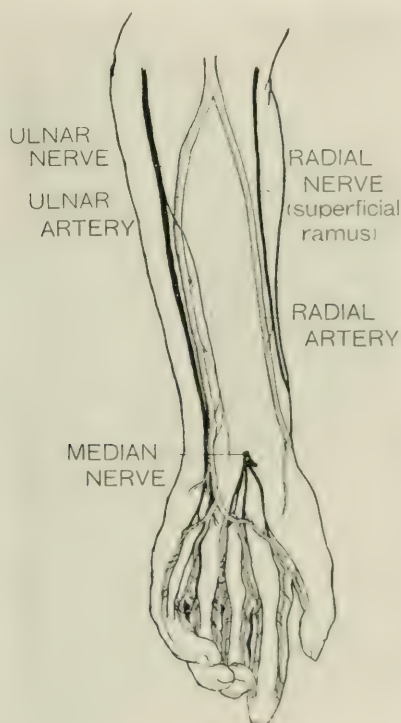


Fig. 3 Diagram to illustrate the nerve-supply to the radial and ulnar arteries and to the superficial volar arch. The radial artery, though sometimes receiving a branch from the musculo-cutaneous nerve, obtains branches from the superficial terminal ramus of the radial nerve. The ulnar nerve supplies its companion artery by twigs from its main trunk and from its palmar cutaneous branch. The complicated supply from median and ulnar nerves to the superficial volar arch and digital vessels is also shown.

It is to be remembered that, as in figure 2, the radial artery near its origin may receive a branch from the musculo-cutaneous nerve.



The ulnar artery obtained its supply from the ulnar nerve in the forearm, several twigs arising from the palmar cutaneous branch (fig. 3).

The superficial volar arch and the digital vessels received a complicated supply from the median and ulnar nerves (fig. 3).

It will be observed that the distribution of nerves to vessels corresponds fairly exactly with the nerve-supply to the skin of the fingers. Also there is a much more plentiful supply of twigs to the vessels in the more distal parts of the limb.



Fig. 4 Diagram of the branches of the ramus profundus of the ulnar nerve to the deep volar arch.

The deep volar arch and its branches received their supply from the deep branch of the ulnar nerve (fig. 4).

The portion of the dorsal carpal arch associated with the little and ring fingers was supplied by the dorsal cutaneous branch of the ulnar nerve but the major portion of the arch, as already mentioned, received its supply from the superficial ramus of the radial nerve (fig. 5).

I was unsuccessful in identifying nerve fibers to the volar interosseous artery and other minor vessels which I have not named.

This, however, does not imply that no nerves are given off to these vessels. Had time permitted the making of a greater number of dissections, filaments undoubtedly would have been ultimately isolated in some instances.

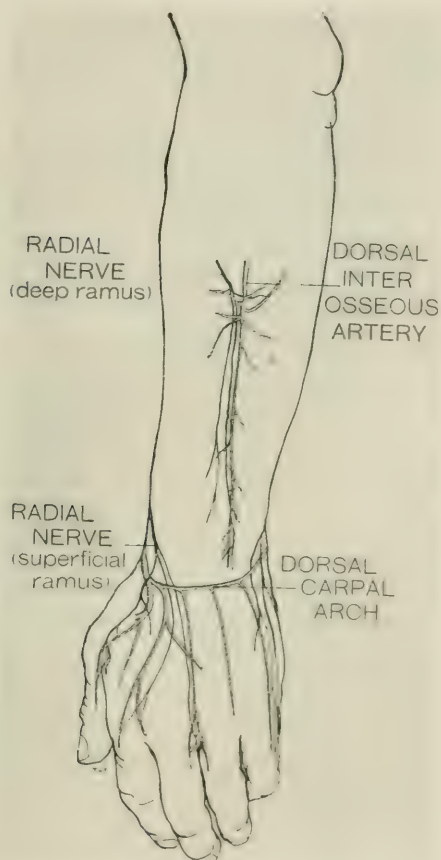


Fig. 5 Diagram to show the distribution of branches from the deep terminal ramus of the radial nerve (posterior interosseous, to the corresponding artery, and of the radial and ulnar nerves to the arteries on the back of the hand.

On the back of the forearm several branches were distributed from the dorsal interosseous nerve (deep ramus of radial nerve) to the dorsal interosseous artery and its branches (fig. 5).

Although the number of limbs dissected was not large, it was sufficient to show that while considerable individual variation exists in the precise situation and origin of the nerve-supply of any given vessel, yet the general vascular nervous supply follows a certain well-defined plan. The proximal vessel of the limb (subclavian artery) receives its nerve-supply directly from the sympathetic chain. The more distal arteries are supplied by sympathetic fibers which have traveled to their distribution along special nerve-trunks and not along main vessels. These twigs are distributed to the vessels from the nerve-trunks at intervals; the intervals growing shorter as the more distal portions of the limb are reached, as though a greater wealth of nerves was needed in these parts. Possibly the diminishing size of the member and consequently the greater need for constant regulation in size of vessels may be associated with this fact. Again the distribution of nerves to vessels corresponds pretty closely with the distribution of nerves to the skin and musculature of the same area.

### III. DISCUSSION OF RESULTS

T. WINGATE TODD

That the nerve-supply of the bloodvessels presents considerable clinical significance has been illustrated by recently recorded cases of cervical rib (e.g., 10). The clinical appearance of the vessels in this disease has been noted in many cases by various writers. Hence it is possible to give briefly the characteristics.

Vascular symptoms commence in the fingers and spread upwards into the forearm: the radial or ulnar arteries or both may be entirely obliterated. The obliteration may proceed with considerable rapidity up the brachial artery. Occasionally the obliteration reaches the subclavian artery, as in the case of C. F. L., described by Keen (11). The rapidity of the process is illustrated by the following notes of a case in a single woman twenty-nine years of age, which came under my observation last year.

Exfoliation by dry gangrene of the tip of the right index finger occurred on July 4, 1912. By the third week in August the pulse could no longer be felt at the elbow. On September 19



the pulse ceased at the level of the insertion of the *M. coracobrachialis*. The pulse in the brachial artery became weaker until September 26, but could still be felt below the posterior fold of the axilla and the pathological change in the arterial wall remained stationary from that date.

The subclavian artery beyond the *M. scalenus anterior*, if not obliterated, appears frequently dilated. This was so in cases recorded by Keen (11) and Hamann (12).

The interference with the circulation in the large vessels is not shared by the smaller arteries in every instance. In Keen's case, C. F. L., the circulation in the arm generally was unaffected in spite of the fact that no pulse could be detected in any of the large vessels from the subclavian artery to the wrist (11). In one case of mine mentioned recently, pulsation could distinctly be felt in some small arteries, although absent in the larger ones (13). This curious phenomenon has been noted experimentally by Lapinski (14), while the lack of tone in the subclavian and axillary vessels referred to above has been shown by Cehanović to occur in experimental lesions (15). The delay of the pulse so often noted in the disease was found by the latter author to be due to the lack of tone in the vessel wall. As regards the early stage of the disease, few exact observations have been made. The clearest statement is that of Osler (16). This author found that in one of his cases the radial pulse on the two sides seemed normal and equal during rest. After some exertion the pulse on the affected side (right) became very small, only just perceptible in fact; the arm becoming congested and cyanotic.<sup>1</sup> As the fallacy of the accepted views on delay in pulse wave and interference with circulation generally, has previously been shown (9), it simply remains for me to state the view to which Mr. Kramer and I have arrived by our present study of the anatomical facts.

The subclavian artery, being supplied directly by a sympathetic twig from the region of the *ansa subclavia*, is but rarely and secondarily affected in the disease known as 'cervical rib.' This twig is not caught in the lesion because it lies alongside the

<sup>1</sup> In a recent personal communication, Sir William Osler confirms this statement and assures me that the fact was noted by many observers in this case.

artery which is not locally damaged. The filaments passing to the more distal vessels, especially to those of the fingers, become affected in certain instances. For precise information regarding the anatomical facts bearing on this point, reference must be made to previous papers from this laboratory. From a study of such cases as that of Osler's, already mentioned, the lesion would seem at first to produce stimulation of the vaso-constrictor nerves, the pulse becoming temporarily small and barely perceptible. Later, paralysis of the vasoconstrictors is indicated by the dilatation and lack of tone with consequent delay in the pulse shown by Čechanovič's experimental work. This dilatation is sometimes apparent in the axillary and distal subclavian arteries in advanced cases. Following on the damage to the nerves, changes occur in the vessel wall which result in obliteration of the lumen and transformation of the artery into a fibrous cord. This action is selective. For just as some muscles escape, certain vascular areas remain unaffected and, in many cases, carry on the circulation in the limb. The reason for this selective action is not yet apparent. As already indicated, there is a tendency for large vessels to be affected and for the smaller ones to escape. Doubtless the cause of this will be found in the distribution of the precise fibers to the several vessels. But at present no definite statement can be made. There is certainly a kind of plexus formation throughout the length of the limb, produced by the interchange of vascular filaments from one large nerve-trunk to another. Exceedingly significant, when one considers the origin and distribution of vascular nerves, is the observation of Weir Mitchell (19) that 'trophic' changes are most prone to follow wounds of the nerves to hand or foot (i.e., lowest cord of brachial plexus in which the majority of the vascular nerves exist) and more rarely occur when the injury has involved nerve branches which supply the upper portion of a limb (p. 38). A striking instance is cited in Case 29 of Weir Mitchell's series (p. 167).

The veins are also affected in the disease, but they lie outside the scope of the present paper, and will be considered on some future occasion.

The practical bearing of the anatomical research at present being prosecuted on the subject of nerve-supply to bloodvessels has been convincingly shown by my erstwhile colleague, Mr. E. D. Telford, in a recent paper in which are to be found details of two cases of neuro-vascular derangement consequent on the presence of cervical ribs, which were subjected to operation with the express purpose of arresting a progressive mechanical lesion of the vascular nerves (20).

Not only was the condition relieved by operation but the symptoms produced by the lesion completely disappeared. In Case II, from which we took a short length of the radial artery, the pulse has not reappeared in the main vessels, but in Case I the radial pulse first returned four months after the operation. Only after our experimental work is finished can we state what happened to the artery in order that the pulse might return. The vessel may have been canalized or a new artery may have grown in the substance of the obliterated vessel, as occurs in the uterus after child-birth.

It is certainly a suggestive fact that the pulse should return four months after operation, when one associates this with the observations of Head and Rivers, who state that the vascular condition of the limb commenced to return to the normal, in their experiment, in 107 days and was quite normal in 190 days after section and primary suture of the superficial ramus of the radial and of the musculo-cutaneous nerves at the level of the fossa cubitalis (21).

In conclusion, it is necessary to point out that a study of the neuro-vascular arrangements in the arm has led me entirely to alter my views from those which I held in 1911, on the causation of the vascular phenomena in 'cervical' rib cases.

For an account of the circumstances leading to this change of view, Dr. Wood Jones' paper before the Royal Society of Medicine, may be consulted (17). My present views on the subject lead me to submit an entirely different description of the method of causation of the pulse characteristics from that given some years ago by Babcock (18).



## IV. SUMMARY

1. The subclavian and axillary arteries differ from the other arteries of the arm in receiving a nerve-supply direct from the sympathetic chain.

2. All other arteries in the upper limb obtain their nerve-supply from sympathetic filaments which have traveled along the spinal nerves and which are distributed to the various blood-vessels at irregular intervals.

3. The distal and peripheral vessels, more particularly those of the hand, receive nerve filaments at more frequent intervals than do the proximal channels.

4. The distribution of nerves to vessels corresponds roughly with the distribution of nerves to muscles and skin.

5. The fact that the subclavian trunk derives its nerve-supply direct from the sympathetic chain accounts for its escape from involvement in the lesion associated with the disease known as 'cervical rib.'

6. Paragraph 4 explains the early involvement of the arteries of the hand in the type of cervical rib lesion in which the vessels are affected.

7. The process of the bloodvessel affection appears to be (a) stimulation of vasoconstrictor fibers, (b) paralysis of vasoconstrictors (with undisturbed action of vasodilators ?) (c) pathological changes in the vessel wall consequent on the lesion of the nerves.

8. For the site and cause of the nerve lesion previous papers from this laboratory must be consulted.

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## PRELIMINARY NOTICE

### International Congress of Anatomy

The International Committee for the International Congress of Anatomy has decided that the next meeting shall be held at Amsterdam during August, 1915. Formal announcement, giving the exact date and other details, will be issued later and distributed to the members of the American Association of Anatomists. At the last Congress, held in Brussels in 1910, there was a good attendance of American Anatomists, and it is hoped that the American representation will be even larger at Amsterdam.



# THE BRAIN OF A BLACK MONKEY, *MACACUS MAURUS*: THE RELATIVE PROMINENCE OF DIFFERENT GYRI

HARRIS E. SANTEE

*From Laboratories of Jenner Medical College and The Chicago College of Medicine and Surgery*

## FOUR FIGURES

This brain measures 7 cm. from frontal to occipital pole. Its greatest transverse measurement is 6.2 cm.; and 4.5 cm. is the height of a cerebral hemisphere. The entire weight equals 114.95 grams, of which the cerebrum comprises 103.29 grams and the rhombencephalon 11.66 grams. The measurements are approximately proportional to the corresponding dimensions of the human brain; but the relative weight of the cerebrum is nine-tenths of the whole brain. In man the cerebrum constitutes seven-eighths of the brain.

Upon examining this brain, one familiar with the human brain is impressed with the probable significance of three facts, namely, a remarkable shortening of the frontal region, an abundant fullness of the occipital and hippocampal regions, and a marked prominence of the central gyri. The central sulcus of Rolando is far from central in position; it divides the convex surface, above and behind the lateral fissure of Sylvius, very nearly into an anterior third and a posterior two-thirds, a notable variation from the proportions of the human brain.

These variations from the human proportions should be in perfect harmony with the widely different psychic functions performed by the brains of man and monkey. Moreover, the high development of the motor and sensory nerves of the monkey and their very strong resemblance to the peripheral nervous

system of man are facts fully as impressive as the monkey's deficiency in psychic apparatus; they speak for the harmony and unity of creation.

Placed alongside the human brain and viewed in the light of cortical localization, a study of the convolutions and sulci of this brain from the *Macacus maurus* possesses something more than its own intrinsic interest.

The central sulcus (Rolandi) is well developed but shortened so that it fails to cut the supero-medial border of the hemisphere (figs. 1 and 3). It possesses two well marked genua and is bounded by two prominent gyri, the anterior and posterior central, which unite around its extremities.

Below the central sulcus and forming a right angle with its inferior extremity, is the great fissure of the convex surface, the lateral fissure of Sylvius. This fissure is unbranched, there being no anterior rami, and is a single fissure from its beginning in the fossa lateralis, near the optic chiasma, to its posterior termination in the concavity of the supramarginal gyrus. Its extremity, which is not bent upward as in the human brain, appears to open into the superior temporal sulcus; but in reality a submerged part of the supramarginal gyrus separates it from that sulcus. The prominence of the anterior parietal region appears to account both for this crowding downward and straightening of the fissure and for the partial submergence of the supramarginal gyrus. The absence of the anterior rami of the lateral fissure is to be expected in view of the fact that the lateral fissure is formed by the excessive growth and the consequent closing in of the walls of the lateral fossa; and, because the frontal wall does not undergo this redundant growth, the anterior rami of the fissure are not produced.

The frontal lobe is deficient anterior to the precentral sulcus. The anterior central gyrus is very large and definite. But the superior, middle and inferior frontal gyri are poorly developed; they are greatly shortened anteriorly, and taper toward the frontal pole.

The inferior frontal gyrus has neither the triangular nor the orbital portion, seen in the human brain; it is flexed over the

orbitofrontal sulcus; at the base, it joins the middle frontal gyrus and, at the anterior end, it is continuous with the superior frontal. The middle and superior frontal gyri are imperfectly separated and form a triangular field with its apex at the frontal pole.

These facts show, first, a large deficiency of psychic brain, as one would anticipate in the monkey; and, second, a relative redundancy of motor brain, which is in keeping with the monkey's enormously developed musculature.

The parietal lobe is large. It is subdivided by an interrupted postcentral sulcus and a very deep horizontal sulcus. The latter begins as inferior postcentral sulcus and winds over the summit of the angular gyrus where it opens into the occipitoparietal; it separates the supramarginal and angular gyri, which are continuous superficially, from the superior parietal gyrus. The superior postcentral sulcus is parallel with the central sulcus as in man, and it intervenes between a well formed posterior central gyrus and a triangular superior parietal gyrus.

The parietal gyri are fully as prominent as in man with the exception of the posterior link of the supramarginal gyrus. This is somewhat suppressed and forms a buried gyrus closing the lateral fissure and connecting the supramarginal with the superior temporal gyrus. The angular gyrus is a large and sharply flexed convolution bent over the angular portion of the superior temporal sulcus, behind which it descends obliquely in front of the simian sulcus (affenspalte) almost to the inferolateral border of the hemisphere; it becomes continuous with both the middle temporal and the lateral occipital gyri, as in the chimpanzee.

In Dwight's chimpanzee the superior postcentral sulcus is longitudinal in direction and divides the superior parietal gyrus into two sagittal gyri, while the horizontal sulcus opens posteriorly into the simian sulcus (affenspalte) instead of into the occipitoparietal sulcus.

The prominent development of the whole parietal lobe is entirely consonant with the fact that this lobe contains the receptive and interpreting centers of common sensation.

The *Macacus maurus* has opposable thumbs and great toes and perfect prehension in all four hands, which are significant



facts in the presence of a well developed praeuneus and superior parietal gyrus, the cortex containing the stereonogistic center.

Alongside the highly developed motor and common sensory gyri, the anterior and posterior central, we should place another remarkable fact, namely, the *enormous peripheral nerves*, which are almost equal in diameter to the nerves of man, though this monkey measures from crown to ischial callosities less than 61 cm. (24 in.) and its entire weight is not over one-fifth that of an average adult of 150 pounds.

The temporal lobe appears large in its convex exposure at first glance, but upon examination this is found to be caused by the crowding of the fusiform gyrus outward into the infero-lateral border of the hemisphere. The superior temporal gyrus is of large size, especially at its polar end. It presents on its superior surface, well back toward the posterior end, one transverse temporal gyrus (of Heschl). The middle and inferior temporal gyri are fused into one and together form a gyrus no larger than the superior. This fused temporal gyrus is divided posteriorly by the inferior occipital sulcus and thus becomes continuous with angular and fusiform gyri.

The superior temporal sulcus is a remarkable one. It extends without interruption from the temporal pole far up into the parietal lobe, showing no sign of separation between the temporal and angular parts. This does not agree with the supposed development of this sulcus in man from two short furrows, a temporal and an angular, which later in uterine life run together. The junction with the lateral fissure is only apparent, the submerged link of the supramarginal gyrus really separates them. The slight indentation below the union of the temporal and angular parts probably represents the descending branch of the superior temporal sulcus as seen in the orang and the chimpanzee.

The satisfactory representation of the receptive acoustic region, the transverse and superior temporal gyri, should be noted in this brain. But no explanation for the bulky anterior end of the superior temporal gyrus is suggested by the facts at hand. If the centers of 'intonations' and 'naming' occupy the anterior two-fourths of the middle and inferior temporal convolutions in man,

we should expect just such suppression of these gyri in the monkey as this brain presents: such intellectual processes as the accurate recognition of tone, pitch and concord appear to have no place in the mentality of the monkey, and Adam only was enjoined to bestow names upon the various objects in his environment; but on the other hand, the reduction in the posterior two-fourths of these gyri, though partly accounted for by the diminutive psychic auditory requirements, is, nevertheless, very strong evidence against the suggestion made by Mills and others, that in man these parts contain the centers of orientation and equilibrium, since the monkey's sense of direction and sense of equilibrium are certainly equal to man's.

The occipital lobe is remarkable for its size and its boundaries; indeed it protrudes so as to appear folded upward over the end of the parietal lobe. The occipitoparietal sulcus which intervenes between them is thus flexed upward (fig. 3). The occipitoparietal sulcus is apparently continued downward on the convex surface almost to the inferolateral border of the hemisphere, the arcus occipitoparietalis being entirely absent (fig. 1). This apparent extension, however, is really a distinct sulcus characteristic of the simian brain, called the simian sulcus (*affenspalte*); it is separated from the occipitoparietal sulcus by two interlocking gyri profundi, which connect the angular gyrus with the superior occipital. The lateral occipital and the inferior occipital sulci are well developed. They run at right angles to the sulcus simialis and have the same arrangement as in the *Macacus sinicus* of Swinington. The lateral occipital sulcus does not reach the sulcus simialis, therefore, between them the triangular superior occipital gyrus joins the anterior end of the elongated lateral occipital convolution. The inferior occipital sulcus lies below the lateral extremity of the simian sulcus, the two being separated by an arcus occipitotemporalis. What appears to be a third or inferior occipital gyrus is in reality the gyrus fusiformis crowded out into the border of the hemisphere.

The location of the receptive visual center almost wholly on the convex surface of the monkey's brain, excepting the lemur and marmoset, explains the great expansion of this part of the

occipital lobe. This monkey possesses binocular vision and has a highly developed visual apparatus.

On the medial surface of the hemisphere the occipitoparietal sulcus fails to join the calcarine fissure and in this respect it appears to agree with all the higher primates below man, though there is a communication between them in Dwight's chimpanzee. The ventral end of the occipitoparietal sulcus is bent sharply upward and then continued forward to the sulcus of the corpus callosum, thus partly isolating a small retro-callosal field in which Flechsig has located the center of taste. It would be interesting to know whether this callosal end of the sulcus is a real sulcus limitans for the gustatory cortex.

Viewing the medial and tentorial structures in the occipital region (fig. 3), we note first an unbroken calcarine fissure with its posterior end turned upward instead of downward. It runs parallel with the occipitoparietal sulcus. It is bifurcated posteriorly and both branches end in the medial surface. Below the calcarine fissure are the sulcus of the gyrus lingualis and the posterior part of the collateral fissure. Both of these open into the calcarine fissure in the right hemisphere (fig. 3), but neither does so in the left. The collateral fissure is deep and continuous almost to the temporal pole (fig. 4). The sulcus of the gyrus lingualis is very shallow; it strongly resembles the same sulcus in the human brain, but it is not a sulcus limitans of striate cortex in this brain—in fact no line of Gennari is visible in gross section of any part of the occipital lobe. The lateral and inferior occipital sulci wind around the border of the hemisphere into the medial aspect, as shown in figure 3.

The cuneus in this brain is not wedge-shaped at all. Bounded by occipitoparietal sulcus and calcarine fissure, it is of nearly uniform width and is bent forward near its middle into the form of a capital L, reversed in the right hemisphere. It is directly continuous with the gyrus cinguli; this junction is formed in the human brain by the submerged gyrus cunei, which is here brought to the surface by the expansion and unfolding of the occipital lobe. The cuneus is not connected with the lingual gyrus.



The gyrus cuneo-lingualis, present in the human brain, is entirely wanting.

The lingual gyrus is very broad. It is divided posteriorly by the sulcus of the gyrus lingualis. In the right hemisphere it is shortened and bounded posteriorly by the fusion of the collateral fissure with the calcarine fissure; but on the left side, where these fissures do not fuse, it extends backward to the occipital pole and is bent downward so as to form a rounded prominence. Anteriorly the lingual gyrus is directly continuous with the hippocampal gyrus.

The hippocampal gyrus is of remarkable size. Its boundaries are very definite. The well developed uncinate (pyriform) region, bounded anteriorly by a definite sulcus rhinalis, is in keeping with the location of the center of smell in this area. The enormous lateral expansion of the hippocampal and lingual gyri almost crowds the fusiform gyrus off the tentorial surface (fig. 4).

The subparietal sulcus is very faint (fig. 3, *c*). Sulcus cinguli is well developed above the corpus callosum where it intervenes between the cingulate and superior frontal gyri (fig. 3, *b*); but the rostral portion (*a*) is not continuous with this part, a separation common in the human brain. The parolfactory sulci are very shallow. The posterior and the more definite sulcus (fig. 3, *f*) forms the anterior boundary of a well developed gyrus subcallosus.

The slender superior frontal gyrus, particularly in its anterior portion, and the very bulky gyrus hippocampi and gyrus lingualis are the notable features of the medial and tentorial surface of this cerebral hemisphere. They emphasize the predominance of archipallium over neopallium.

The base of the forebrain presents an orbital surface modeled almost exactly like the human brain, the olfactory and orbital sulci being similar as to situation, length and direction. There is no indication of medial and lateral olfactory gyri seen in a six months human embryo. Three things are especially noticeable in the basal surface. First, the mammillary body is median and single, as it is in the human embryo and in the adults of many

lower animals. Second, the presence of the gyrus cunei on the surface, connecting a very narrow cuneus with the gyrus cinguli. The third is a fact already mentioned but further emphasized in this view of the cerebrum, namely, the massiveness of the hippocampal gyrus, especially of its pyraform area, the uncus hippocampi. So, the cortical center of smell, like all other sensory centers, is abundantly represented in this brain of the *Macacus maurus*.

#### SUMMARY

*All parts of this brain which are sensory or motor in function are largely developed, while there is some deficiency in the posterior parietal and inferior temporal regions and remarkable defect in the frontal lobe anterior to the precentral sulcus. These deficiencies lie within the association areas of Flechsig, as was to be anticipated in the beginning.*

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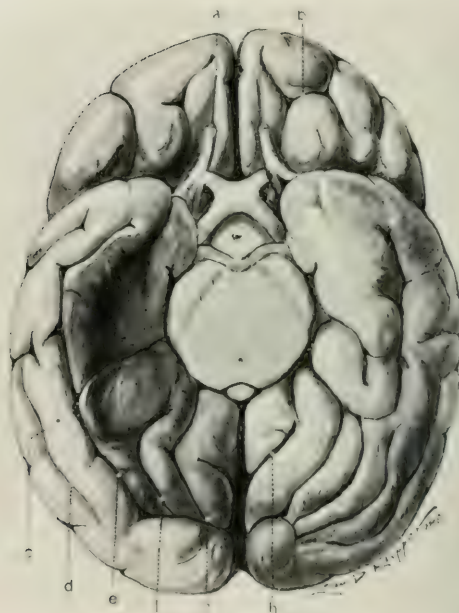
Fig. 1 Convex surface of the left cerebral hemisphere of the *Macacus maurus*. *A, c*, sulcus frontalis inferior; *b, d*, *s. frontalis superior*, broken into two parts; *e*, *s. praecentralis inferior*, continuous above with superior frontal sulcus; *f*, *s. praecentralis superior*; *g, h*, *s. postcentralis inferior*; *g*, continued as *s. horizontalis*; *i*, *s. centralis* with genu superius and genu inferius opening backward; *j*, *s. postcentralis superior*; *k*, *s. occipitoparietalis*, which receives the horizontal and on the surface is continuous with the *s. simialis*. The latter runs obliquely downward and forward toward the infero-lateral border of the hemisphere (*Affenspalte* or *s. lunatus*); *l*, *s. orbitofrontalis*; *m*, *fissura cerebri lateralis* (*Sylvii*); *n*, *s. temporalis superior*; *o*, *s. temporalis inferior*; *p*, *s. occipitalis lateralis*; *q*, *s. occipitalis inferior*.

Fig. 2 *Insula* (*Reilii*), the frontal, parietal and temporal opercula being cut away. *A*, anterior lobule of insula made up of four very rudimentary gyri breves; *b*, posterior lobule of insula, *gyrus longus*; *c*, *sulcus centralis insulae*.





3



4

Fig. 3 Medial surface of right cerebral hemisphere of the *Macacus maurus*. *A*, sulcus rostralis, or the anterior part of the cingulate sulcus; *b*, *s. cinguli*; *c*, *s. subparietalis*; *d*, *s. occipitoparietalis*; *e*, fissura calcarina at its posterior end where it bifurcates; *f*, *s. parolfactorius posterior*; *g*, *s. rhinalis*, very well developed; *h*, a slot leading to the fissura chorioidea, commonly called hippocampal fissure; *i*, *f. collateralis*; *j*, *s. sagittalis gyri lingualis*.

Fig. 4 Base of forebrain of the *Macacus maurus*, the midbrain being cut through transversely. *A*, sulcus olfactorius and a part of the olfactory tract; *b*, *s. orbitalis*, H-shape; *c*, gyrus fusiformis; *d*, *s. occipitalis inferior*; *e*, fissura collateralis; *f*, *s. sagittalis gyri lingualis*; *g*, *f. calcarina*; *h*, *s. occipitoparietalis*, inferior end.

## AN ADDITIONAL CASE OF PANCREATIC BLADDER IN THE DOMESTIC CAT

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ONE FIGURE

In The Anatomical Record for 1911, Dresbach describes the sixth case of pancreatic bladder in the domestic cat that has been recorded in recent years. Miller ('04, '10) had previously reported and described five pancreatic bladders from his laboratory, but prior to his accounts only two such anomalies were on record, one by Mayer in 1815, the other by Gage in 1879. It appears from a footnote in Dresbach's article that Miller has more recently found two additional cases of pancreatic bladder, making a total of seven from his laboratory.

As suggested by Dr. Miller ('10), it would appear that either there existed a breed of cats in his locality among which pancreatic bladders were of relatively frequent occurrence, or else there had been careless observation in other laboratories where cats are generally used for dissection. The fact that three of the cases described by him occurred in cats from the same farm-house, two of which were full brothers, strongly supports the former alternative, and that the structures were inherited. From experience in the laboratory at Minnesota, I am of the opinion that pancreatic bladders are generally rare.

The case here reported was found in this laboratory a number of years ago, 1906 or 1907, by Dr. John C. Brown, of St. Paul, then in charge of the courses in vertebrate anatomy, who later kindly left the specimen at my disposal. Following the finding of this pancreatic bladder it was made a practice to caution all students in cat dissection to examine their specimens care-

fully for further occurrence of such structures, and in practically every instance the cats were also examined by Dr. Brown and myself. However, in the five or six years that have elapsed, no additional cases have appeared. During this time approxi-

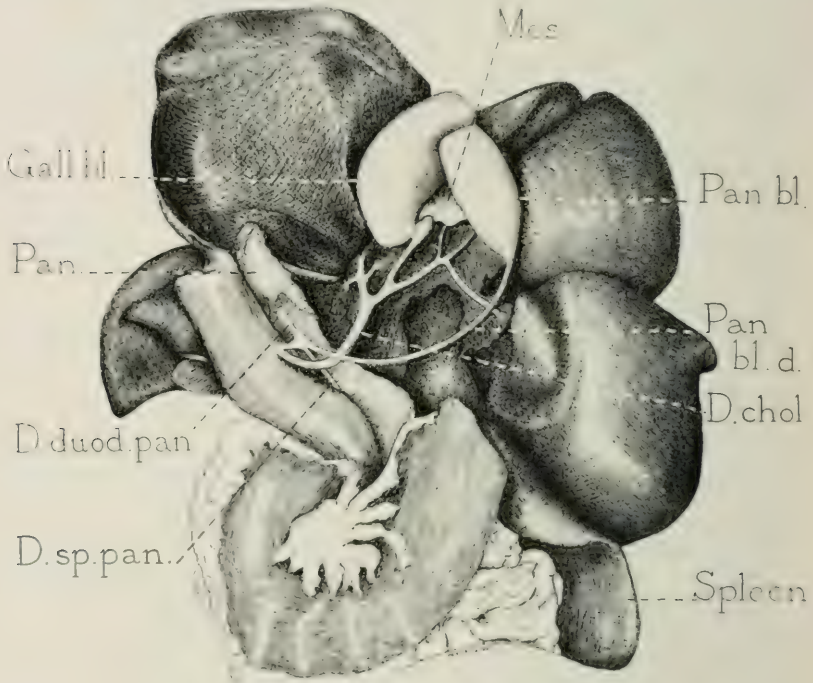


Fig. 1 Drawing showing pancreatic bladder. The liver is represented as seen from the dorsal side when its free edge has been lifted and thrown forward. *D. chol.*, ductus choledochus; *D. duod. pan.*, duct from the duodenal portion of the pancreas; *D. sp. pan.*, duct from the splenic portion; *Gall. bl.*, gall-bladder; *Mes.*, mesentery holding pancreatic bladder; *Pan.*, pancreas; *Pan. bl.*, pancreatic bladder; *Pan. bl. d.*, duct of pancreatic bladder.

mately four hundred cats have been dissected in the laboratory; and the probability that pancreatic bladders have escaped our notice, I believe, is exceedingly small. Our cats have come from various parts of the city of Minneapolis but probably a majority from the same section.



## DESCRIPTION

The liver, with the stomach and a portion of the duodenum attached, had been removed from the body before it came into my hands. Surrounding tissues had been dissected away and the ducts of the liver and the pancreas exposed. The duodenal division of the pancreas had in large part been cut away, but leaving the portion adjacent to the ampulla of Vater intact. The whole had been preserved in formalin.

The pancreatic bladder is of a type similar to that represented by Miller's ('04) Case I. The gall-bladder lies in the usual position, however, and the pancreatic bladder is relatively smaller, being approximately a half or two-thirds the size of the gall-bladder. It is situated on the left of the gall-bladder and is attached to the dorso-caudal surface of the quadrate lobe of the liver by a fold of peritoneum, forming a sort of mesentery about half an inch in width, which extends from the fundus of the bladder to within a short distance of its opening into the duct. The ductus choledochus and hepatic ducts show normal conditions. In its present condition the duct of the pancreatic bladder is about 40<sup>mm.</sup> in length, and leaving the bladder, passes in a curve from left to right, crossing the ductus choledochus, and enters the ductus pancreaticus at the junction of the ducts from the duodenal and splenic divisions of the pancreas. Thus the collecting ducts of the pancreas and the duct of the pancreatic bladder meet at a common point to enter the ductus pancreaticus. A slight dilatation occurs at this junction, but hardly deserving the name sinus. The relation of the duct of the pancreatic bladder to the duct of the duodenal and of the splenic portion of the pancreas is in this particular slightly at variance with the conditions in other described cases, where the duct from the bladder connected either with the duodenal branch of the ductus pancreaticus, with the splenic branch, or with both splenic branch and ductus pancreaticus. The ductus pancreaticus enters the ampulla of Vater in the usual way, alongside the ductus choledochus.

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ON THE RELATIVE GROWTH OF THE ORGANS AND  
PARTS OF THE EMBRYONIC AND YOUNG  
DOGFISH (MUSTELUS CANIS)

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CONTENTS

Introduction.....	271
Materials and methods.....	272
Discussion of data.....	275
Growth of head.....	275
Growth of systems (skin, skeleton, musculature and viscera as a whole)	277
Growth of individual organs.....	280
a. Brain.....	280
b. Spinal cord.....	281
c. Eyeballs.....	281
d. Heart.....	282
e. Pancreas.....	283
f. Liver.....	283
g. Spleen.....	285
h. Rectal gland.....	285
i. Kidneys.....	286
j. Gonads.....	286
k. Stomach-intestines.....	287
Summary.....	288
Literature cited .....	291

INTRODUCTION

In general, but little attention has been paid to the relative growth of the various organs in fishes. Some scattering observations are included in the work of Welcker and Brandt ('03). The most complete data are those of Kellicott ('08) on the dogfish. These, however, deal only with the relations from birth onward. A knowledge of the earlier embryonic conditions is very desirable in order to trace more completely the growth process and to give a more extended basis for comparison with



higher vertebrates. Therefore the purpose of the present paper is to present and discuss some original observations on the relative growth of the viscera and parts of the embryonic dogfish. Some original data on the postnatal relative growth of the young dogfish are included for comparison with embryonic growth and with postnatal data already in the literature. The relative growth of the dogfish is briefly compared in a general way with the data in the literature on the relative growth of higher vertebrates, especially mammals.

This work was done in the Anatomical Laboratory of the University of Missouri under the direction of Dr. C. M. Jackson, to whom I am deeply indebted for invaluable criticism and suggestions.

#### MATERIAL AND METHODS

##### *a. Species examined and description of serial weights and lengths*

The materials used in this paper consisted of 47 dogfish (*Mustelus canis*). The dogfish ranged in weight from 0.0084 gram to 1498.8 grams, and in length from 16 mm. to 800 mm. Of the embryos, 15 weighed less than 1 gram, two between 1 and 2 grams, one between 2 and 3 grams, six between 2 and 4 grams, three between 4 and 5 grams, and two weighed a little more than 5 grams. There is a gap in the series between the 5-gram embryos and the fish at or a little before birth, the next larger fish weighing 42.075 grams. Three fish weigh between 40 and 50 grams, two between 50 and 60 grams, one 65 grams, one (approximately) 72 grams, one 82 grams, one 92 grams, and four between 100 and 133 grams. Here again there is a gap, the next larger fish weighing 331 grams. Four fish weigh between 300 and 400 grams, and the largest of the series 1498.8 grams. Twenty-one of the dogfish were males, seventeen were females, and in nine the sex could not be ascertained. Sex was determined by the presence or absence of claspers, which structures are present in the male of the species as a partly detached portion of the medial edge of the ventral fin. These dogfish were obtained from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts.

*b. Preservation of the specimens*

The larger dogfish (42 grams and up) were preserved in 5 per cent formalin. Formalin causes in general a swelling of the tissues, which, from data by Jackson ('09), amounted to nearly 13 per cent of the total volume of a human fetus of the fifth month, after three months' immersion in 10 per cent formalin solution. Formalin is also known to cause unequal expansion of the tissues which fact must be recognized as a source of error in relative weights of a specimen preserved in formalin. It is unlikely, however, that error from this source would be great enough to influence materially the general conclusions regarding relative growth. The embryonic dogfish were fixed in mercuric chloride and preserved in alcohol. Alcohol causes shrinkage in the tissues, and moreover, being very volatile, would give rise to error from evaporation. To offset this, the alcoholic specimens were soaked in water for several days before weighing. The replacement of the alcohol by water in addition to diminishing the error by evaporation, also caused the tissues to swell and regain to a certain extent their former volumes. On account of the possible changes in the various organs due to the effect of preservatives, allowance must be made for a certain amount of unavoidable error.

*c. Measurements and dissecting methods*

The fish were first washed in water, if formalin-preserved, and then the total length (tip of nose to tip of tail) and trunk length (tip of nose to anus), was carefully noted. Then the gross body weight was taken and the sex noted, after which the organs and parts were dissected out and weighed. Organs not being weighed were kept on a moistened filter paper in a dish with a ground-glass cover. First, the head was separated from the body just behind the mandibular arch, and weighed. Then the eyeballs were removed, the extra-ocular muscles dissected off, and the optic nerve clipped close to the eyeball. The brain was removed by removing the roof of the cranium and cutting the cranial nerves close to the point where they emerge from or enter the

brain. In removing the head the cord was cut across posteriorly at its junction with the brain. The heart was separated anteriorly at the junction of the conus arteriosus and truncus arteriosus, and posteriorly at the junction of the auricle and sinus venosus. Organs having a mesentery were removed by cutting along the line of attachment of the mesentery to the organ. The stomach and intestines were weighed first with contents and then without contents, the difference being subtracted from the gross body weight to give the net body weight. The contents, however, were usually slight in amount. The rectal gland was removed at the line where its duct commenced. The skin includes the skin of the entire body and the adherent subcutaneous tissue. The musculature and the skeleton (minus the few remaining structures, gills, esophagus, etc.) were weighed together, and then the musculature was dissected off and the skeleton and ligaments alone weighed. The difference gives the weight of the musculature. The organs and parts weighed were: brain, spinal cord, eyeballs, heart, pancreas, liver, spleen, rectal gland, kidneys, gonads, stomach and intestines, skin, musculature, and skeleton and ligaments. This list is more extensive than that of Kellicott, who observed only the following organs: brain, heart, pancreas, spleen, liver, gonads and rectal gland. Any apparent abnormalities were noted. In the case of an organ containing cavities, such as the heart, the cavities were thoroughly cleansed before weighing. In the specimens preserved in alcohol the blood in the heart was hardened and sometimes could not be thoroughly removed, which accounts for some of the large variations in the weight of this organ.

In the smaller fish the weights were recorded to the ten-thousandth of a gram (tenth of a milligram), with the exception of a few instances in which the weights were recorded to the one-thousandth of a gram. In the larger fish, weights were recorded to the one-thousandth of a gram, except in the case of total body weights and structures too large to be safely weighed on such delicate balances. As a rule, however, in referring to the body weights in the following paper, the figures are carried only to the first decimal place (tenth of a gram). The percentages are



likewise given, unless it is necessary to carry to further decimal places in order to give two significant figures. The organs were first rolled gently on filter paper to remove superfluous moisture and were then placed in a closed dish for weighing to prevent loss by evaporation.

#### DISCUSSION OF DATA

To reduce the range of individual variation and thus give a more nearly correct idea of the average relative size of the parts and organs at various periods, figures representing the averages of several individuals of approximately the same body weight are used largely in the discussion instead of considering each individual separately. The individual data are included in the general table at the end of the paper.

The group of dogfish whose body weights range between 42 and 82 grams, are considered to represent approximately the conditions found at birth. All these fish appeared to be free living. Kellicott found the average weight of 13 dogfish at birth to be 76.2 grams (maximum 84, minimum 69.5). These fish were born of a single female weighing 8434 grams and this also is the maximum number of young recorded for this fish; so Kellicott says the weights may not represent the precise conditions found at birth.

##### *1. Growth of the head*

An inspection of the general table of observations shows, as has been repeatedly observed in many different species of animals, that the head is relatively largest early in embryonic life. In the series of fish under discussion, three dogfish embryos with an average body weight of 0.0346 gram have an average percentage head weight of 26 per cent. In an average of three slightly larger fish weighing about 0.0854 gram the average percentage head weight is 38.9 per cent, in one case reaching 40.5 per cent. The percentage now decreases, at first sharply and then more gradually, to 19.5 per cent in an individual with a body weight of 2.6 grams. From here it rises unexpectedly to 23

per cent in an average of six specimens with an average body weight of 3.7 grams. The average relative weight of both the eyeballs and the brain also rises at this point. The percentage weight of the head now drops to an average of 21 per cent in five individuals having an average body weight of 5 grams. Thus on the whole, the relative weight of the head in this series of dogfish becomes less as the fish increases in body weight.

In the case of the young dogfish, the table of averages indicates that in general, the percentage weight of the head decreases with age. The variations that exist are small. The percentage weight at about birth is about 17.5 per cent. From this figure, the percentage weight drops rapidly to 12.1 per cent in an average of four individuals with an average body weight of 347 grams. The fluctuations that exist probably represent individual variations. The largest fish weighed 1499 grams and had a percentage head weight of 12.9 per cent. Kellicott's ('08) data do not include the head.

From the above series of data it is evident that the percentage weight of the head is highest in the early stages of embryonic growth; that in general it may be said that the head becomes relatively smaller with age. Jackson has found this true in the human embryo, the head reaching its maximum relative size of about 45 per cent of the total body volume during the latter half of the second month. Lowrey finds the same thing true in the pig, the 18 mm. pig having a relative head weight of 29.69 per cent. The relative head weight of the pig thereafter decreases to 6.26 per cent of the total body weight in the adult. The adult human head forms about 6 to 9 per cent of the total body weight. The adult dogfish apparently has a percentage head weight of about 12 per cent. Jackson and Lowrey have observed in the white rat that the head increases in relative size shortly after birth, reaching its maximum (postnatal) in the second week. So far as I have been able to ascertain, this is the only species in which this has been observed.

## 2. *Relative growth of systems*

*a. Skin.* Dissection of the skin in small fish embryos is difficult, and the liability of error greater than in some of the other measurements.

From the data of the general table, it is evident that the growth of the skin is rather variable. In 20 embryos up to 5 grams, it forms an average of 6.7 per cent of the total body weight (range 5 to 8.7 per cent), with no distinct change according to age.

In 13 young dogfish from 43 to 133 grams, the percentage weight of the skin averages 11.3 per cent (7.4–14.5 per cent). Then it drops somewhat gradually to 7.3 per cent in the fish weighing 1499 grams.

*b. Skeleton (including cartilages and ligaments).* The smallest dogfish embryo in which the percentage weight of the skeleton was determined weighed 2.6 grams, and the relative weight of the skeleton was 7.8 per cent. In a heavier individual with a body weight of 3.8 grams, the percentage weight of the skeleton was 4.5. Two individuals averaging 5.1 grams in body weight had an average percentage skeleton weight of 5.5 per cent. The data on the embryos are meager and variable, but seem to indicate that the percentage weight of the skeleton is smaller than in the later stages.

In the young dogfish the data on the skeleton are somewhat more complete. In twelve individuals weighing from 42 grams to 133 grams, the average percentage weight of the skeleton was 8.6 per cent, varying from 6.8 per cent to 10.5 per cent. In the five largest fish the average was 9.2 per cent. Individual variations make the general trend uncertain.

*c. Musculature.* Owing to the difficulty of dissecting out the skeleton in such small fish, the musculature and skeleton were weighed together in most of the embryos, and to make the percentages comparable, the percentage weight of the musculature is considered *plus the skeleton* in nearly all the embryos. This makes the figure higher than that for the musculature alone, which must be borne in mind.



In an average of 20 dogfish embryos, the percentage weight of the musculature and skeleton is about 44 per cent. Allowing 6 per cent for the skeleton would leave 38 per cent of the body weight for musculature.

In the young dogfish the musculature is considered apart from the skeleton. In 11 fish ranging in body weight from 42 to 133 grams, the percentage weight of the musculature averages 45.4 per cent. From here it rises rather sharply to 58.1 per cent in an average of 4 fish weighing 347 grams, and then more slowly to 63 per cent in a fish weighing 1499 grams.

From the above data it is evident that, in general, the relative weight of the musculature of the dogfish embryo (for the stages observed) is somewhat smaller than at birth. Also that after birth the musculature tends in general to increase in relative weight. Compared with other animals, the dogfish has a very large proportion of musculature.

In the white rat, Jackson and Lowrey have found that the musculature forms about 24.4 per cent of the total body at birth. This percentage decreases to 22.8 per cent at one week, and thereafter increases to 45 per cent in the adult rat. For the human newborn, Mühlmann estimates the percentage weight of the musculature to be 22.4 per cent, increasing to 43.2 per cent at forty-one to fifty years, and thereafter decreasing to 18.6 per cent in old age. In the dogfish the musculature probably increases in percentage weight throughout life. This is due to the fact that the dogfish apparently does not reach a definite adult condition such as that found in mammals. Growth probably continues (according to Kellicott) until the animal dies. In reptiles, Welcker and Brandt find the percentage weight of the musculature to vary from 19 to 57 per cent, in amphibia, from 43 to 54 per cent and in fishes from 49 to 59 per cent.

*d. Viscera (as a whole).* Under this head are included the central nervous system, thoracic and abdominal viscera. The percentage is computed by adding the percentage weights of the individual organs.

In an average of 3 dogfish embryos the body weight is 0.34 gram, and the percentage weight of the viscera 18.9 per cent.

This percentage rises to 19.2 per cent in two individuals having an average body weight of 0.67 gram. In the average of the next group of two fish, the body weight is 1.44 grams and the percentage weight of the viscera 17 per cent. The next fish weighs 2.6 grams, and the percentage weight of the viscera is 14 per cent. From here the percentage rises slightly to 14.4 per cent in five fishes having an average body weight of 3.7 grams. In the average of the next three fish the body weight is 4.8 grams and the visceral percentage 15.2 per cent. The foregoing figures indicate that the visceral group in the earlier embryos is relatively large, but that it diminishes in relative size as growth progresses. In the earlier embryos the high figure is due to the high percentage weight of the central nervous system at this period. As the central nervous system grows relatively smaller, some of the other organs (principally the kidneys) grow relatively larger but do not counterbalance the drop in the nervous system.

In the young dogfish the percentage weights of the viscera at about birth are somewhat lower than in the embryos observed. In a fish weighing 42 grams the relative weight of the viscera is 12 per cent. In the next larger fish the body weight is 47 grams and the relative weight of the viscera is 13.5 per cent. In an average of two larger fish the body weight is 62 grams and the percentage weight of the viscera 11.5 per cent. The next fish weighs 72 grams, of which weight the viscera form 10.6 per cent (the foregoing fish are at or about birth, and in an average of the entire group the percentage weight of the viscera is 12.1 per cent). In the next group of averages, the percentage weight of the viscera in fishes whose body weights range from 92 to 128 grams remains constantly a little over 12 per cent. In an average of the next group of three fish the body weight is 347 grams and the relative weight of the viscera 14.3 per cent. This figure declines to 9.8 per cent in a fish weighing 1499 grams.

From the foregoing it may be seen that the visceral percentage is relatively high in the earlier stages of the embryo and that it drops as the general growth of the embryo progresses. At birth the percentage has dropped to about 12.1 per cent. In general,

the percentage apparently increases after birth to a certain point (14.3 per cent in 347-gram fish) and thereafter decreases. Percentage weights of the viscera in the adult dogfish are variable on account of the variability of the liver, in which, as Kellicott has observed, the variability is due to the amount of fat present in the organ. In the white rat, Jackson and Lowrey find the visceral percentage at birth to be 18.05 per cent, which figure increases to a maximum of 21.28 per cent at three weeks and thereafter decreases to 13.3 per cent at one year. This corresponds in general with the visceral growth in the dogfish.

### *Relative growth of individual organs*

*a. Brain.* The relative size of the brain is large in the early dogfish embryo, but decreases through embryonic as well as post-natal life. In six embryos having an average body weight of 0.06 gram, the average percentage weight of the brain is 11.4 per cent (range 7.8 to 15.5 per cent). In general, this decreases in the embryos observed at first rapidly and then more slowly to an average of 2.2 per cent in five embryos having an average body weight of 4.9 grams.

At birth the percentage weight of the brain is between 1 and 2 per cent. Kellicott finds it to be 1.116 per cent, which percentage decreases at first rapidly and then more slowly throughout life. Although there are some fluctuations (the percentage weight of 2.2 per cent recorded for an individual weighing 102 grams is probably either an error or an abnormality), my data (for the weights observed) show likewise a decrease. In a fish weighing 42 grams the percentage weight of the brain is 1.9 per cent; in a fish weighing 1499 grams it is 0.42 per cent.

The relative weight of the brain has been more extensively studied than that of any other organ, and in general, has always been found to decrease with increase in body weight. Jackson ('09) finds that in the early human embryo the brain increases in relative weight to a maximum of 20 per cent in the second month, thereafter decreasing to an average of 12.8 per cent in the still-born, and 14.6 per cent in the live-born fetus. Lowrey



finds the brain in the early pig embryo attaining a maximum relative weight of 9 per cent at 18 mm., thereafter decreasing to about 4 per cent at birth and 0.087 per cent in the adult. Jackson ('13) finds the maximum postnatal relative size of the brain in the white rat to occur, not at birth, but a short time later, reaching 6.7 per cent.

*b. Spinal cord.* An inspection of the table of relative growth of the spinal cord of the embryonic dogfish will show that the spinal cord is relatively large in the early embryo. In a fish weighing 0.1867 gram, the relative weight of the spinal cord is 1.76 per cent. From here it falls at first rapidly and then more slowly to an average of 0.24 per cent in five fish with an average body weight of 3.7 grams. It apparently rises finally to an average of 0.34 per cent in four embryos with an average body weight of 4.8 grams.

At some stage between the embryos examined and birth, the relative weight of the cord apparently rises, for in a fish weighing 42 grams it averages 0.50 per cent. As the body weight of the young dogfish increases, the relative weight of the cord fluctuates but on the whole diminishes, and finally drops to 0.17 per cent in a fish weighing 1499 grams.

In the human embryo, Jackson ('09) finds that in the fifth week the percentage weight of the cord is 4.85 per cent, and that it diminishes at first rapidly and then more slowly, to about 0.15 per cent at birth. Vierordt gives 0.18 per cent for the relative weight of the cord at birth and 0.06 per cent for the adult. In the pig, Lowrey finds the relative weight of the cord to decrease from 1.87 per cent at 18 mm. to 0.33 per cent at birth and to 0.04 per cent in the adult. In their observations on amphibia and reptiles, Welcker and Brandt find that the cord approaches or exceeds the brain in relative weight. In the dogfish embryo the spinal cord is usually only about one-tenth as large as the brain, while in the adult it is about one-third as large.

*c. Eyeballs.* In the early dogfish embryo the relative weight of the eyeballs increases rapidly to a maximum of 9.4 per cent in an embryo of 0.06 gram body weight. This percentage drops with irregular variations to an average of 3.6 per cent in four

embryos with body weight of about 5 grams. At birth the eyeballs form about 2 per cent of the body weight.

In the young dogfish, the relative weight of the eyeballs drops from about 2 per cent at birth to 1.1 per cent in four fish averaging 347 grams in body weight. At 1499 grams body weight, the eyeballs form about 0.64 per cent of the body. Throughout post-natal life the eyeballs are as large as the brain and spinal cord combined.

In the pig, Lowrey finds that the eyeballs reach a maximum of 1.15 per cent in relative size when the embryo is 86 mm. in length, decreasing to 0.41 per cent at birth and to 0.011 per cent in the adult. In three human fetuses of about the sixth month, Jackson ('09) finds the eyeballs to form 0.45 per cent, 0.40 per cent, and 0.39 per cent of the total body weight. According to Vicerodt, the eyeballs form 0.24 per cent of the total human body weight at birth and 0.02 per cent in the adult. Welcker and Brandt give data on percentage weight of the (adult) eyeballs showing the following ranges: fishes, 0.17 per cent to 2.52 per cent; amphibia, 0.56 per cent to 0.85 per cent; reptiles, 0.02 per cent to 0.56 per cent. The eyeballs of the dogfish embryo appear to be unusually large in relative size. They are, however, relatively smaller than in the chick. In this animal they reach a maximum of about 25 per cent of the body in the embryo, decreasing to 3 per cent in the newborn, and to 0.3 per cent in the adult.

*d. Heart.* In the two youngest embryos examined, the heart of the dogfish has a percentage weight of 2.4 per cent and 4.3 per cent of the body weight. Earlier stages might show a higher maximum. This high percentage falls rapidly, with irregular variations to 0.21 per cent in four embryos averaging 4.9 grams in body weight.

In the dogfish at birth, Kellicott finds the average relative weight of the heart to be 0.11 per cent. In the series presented in this paper (which are of course subject to variation from the small number of observations) the relative weight of the heart at about birth averages somewhat higher, being 0.15 per cent. This percentage may be higher than Kellicott's, partly from the

fact that he did not weigh the complete heart, but only the ventricle and conus arteriosus. Shortly after birth the percentage weight of the heart rises slightly, reaching a maximum of 0.21 per cent in an individual weighing 92 grams. From here it falls, with fluctuations, to 0.2 per cent in a fish weighing 1499 grams. This last figure is probably too high, since Kellicott, in a larger series, finds the percentage weight of the heart in fish of this size to be 0.087 per cent.

In the early human embryo, Jackson ('09) has estimated the relative weight of the heart to be more than 5 per cent of the total body. He finds that it decreases rapidly and reaches about 0.7 per cent at birth. In the newborn, Vierordt estimates the relative weight of the heart to be 0.76 per cent, and in the adult, 0.46 per cent. Lowrey finds the curve of growth of the embryonic and adult pig heart to be similar to that of the human.

*e. Pancreas.* The pancreas in the embryos weighed was relatively small, forming, in six fish with an average body weight of 0.31 gram, 0.072 per cent of the total body weight. Throughout the series of embryos the pancreas is exceedingly variable. In three averaging 4.9 grams in body weight the percentage weight of the pancreas is 0.064 per cent.

In the young dogfish the pancreas forms at about birth approximately 0.06 per cent of the body weight. In a group of four fish averaging 347 grams in body weight, the percentage weight of the pancreas has increased to 0.14 per cent.

Kellicott finds the percentage weight of the pancreas in the dogfish at birth to be 0.08 per cent; this he finds increasing to a maximum of 0.137 per cent in fish weighing about 200 grams, and decreasing thereafter to about 0.075 per cent. In the human embryo, Jackson finds the percentage weight of the pancreas small at first, being 0.032 per cent in a specimen of the sixth week, while at birth it is 0.145 per cent in the live-born. Vierordt gives 0.11 per cent of the total body weight for the pancreas in the newborn, and 0.15 per cent for the adult. Lowrey finds the curve of relative growth of the pancreas in the pig similar to that of the human.



*f. Liver.* In the earlier stages of embryonic life the liver of the dogfish is relatively small, forming in four embryos with an average body weight of 0.0752 gram but 2.4 per cent of the total body weight. This percentage rises very suddenly to an average of 5.8 per cent in six fish with an average body weight of 0.3109 gram and then falls very gradually to 4.6 per cent in five embryos with an average body weight of 4.9 grams. The maximum observed was 7.7 per cent.

At about birth the average percentage weight of the liver is 4.8 per cent. This is somewhat higher than the figure (3.12 per cent) given by Kellicott. This, of course, may be due to the variations in the smaller number of specimens in this series. After birth the relative weight of the liver apparently rises to a maximum of 6.9 per cent in four fish with an average body weight of 347 grams. In a fish weighing 1499 grams this has decreased to 5.9 per cent of the total body weight.

The relative size of the liver in the dogfish is variable. Kellicott thinks that this variability is due to the presence of fat in the organ. He finds that in livers of high percentage weight the percentage of fat is also higher, and vice versa. In six of the largest dogfish he measured, Kellicott finds the percentage weight of the liver to be 5.5 per cent, considerably more than in the human adult. In the human embryo, figures by Jackson show that the liver attains a somewhat higher maximum of percentage weight than in the dogfish, reaching an average of about 7.5 per cent in the second and third months. At birth the percentage is decreasing but is still higher than in the dogfish, being 5.23 per cent in the live-born (Jackson). In the adult human, Vierordt estimates that the liver forms 2.75 per cent of the total body weight. In the liver of the albino rat, Jackson finds the percentage weight to *decrease* from 4.74 per cent at birth to 3.39 per cent at seven days, thereafter increasing to a maximum of 6.78 per cent at six weeks. This author finds the variability of the liver of the albino rat to be large and irregular. In the pig embryo, Lowrey finds the liver to reach a maximum of 15.88 per cent of the total body weight at 25 mm. Welcker and Brandt give data on the percentage weight of the (adult) liver with

ranges as follows: of fishes, 1.57 per cent to 3.85 per cent; amphibia, 2.63 per cent to 6.79 per cent; reptiles, 3.33 per cent to 5.78 per cent.

*g. Spleen.* In the dogfish embryo the spleen is relatively small, and exceedingly variable, the average in the series of twenty-two embryos observed being 0.049 per cent of the total body (range 0.025 to 0.105 per cent).

At about birth the average percentage weight of the spleen is 0.098 per cent, somewhat lower than that observed by Kellicott (0.126 per cent). This increases rapidly with some fluctuations to 0.378 per cent at 347 grams body weight and then falls more gradually to 0.185 per cent in a fish weighing 1499 grams. The variability of the spleen is striking. In two given individuals of approximately the same body weight the spleen of one may be twice or three times as large as the other. This is in agreement with the great variability of the spleen in higher forms.

In the human, Jackson has found the spleen to increase slowly in relative size up to the seventh month; thereafter it increases rapidly, averaging 0.43 per cent in the live-born. Vierordt gives 0.25 per cent for the spleen in the adult human. Lowrey finds the prenatal growth curve of the spleen in the pig similar to that of the human. In the white rat, Jackson finds the relative weight at birth to be 0.22 per cent, increasing to a maximum of 0.41 per cent at one week and thereafter decreasing. Welcker and Brandt give data on the relative weight of the (adult) spleen with ranges as follows: of fishes, 0.15 per cent to 0.34 per cent; amphibia, 0.05 per cent to 0.28 per cent; reptiles, 0.04 per cent to 0.11 per cent.

*h. Rectal gland.* The rectal gland, like most of the organs, is relatively heavier in the embryo than after birth. The average of twenty-three embryos gives 0.105 per cent of the body weight. The extreme variations (0.031 to 0.239 per cent) are probably due largely to difficulty in dissection.

At about birth the relative size of the rectal gland has diminished to an average of 0.032 per cent of the total body weight. Kellicott's figures place this a trifle higher, 0.0398 per cent. The rectal gland appears to increase slightly in relative size after

birth, reaching 0.046 per cent in a fish weighing 92 grams, and thereafter decreasing gradually to 0.022 per cent in a fish weighing 1499 grams. Kellicott finds no such increase after birth, the curve of percentage weight falling throughout life.

*i. Kidneys.* In the dogfish embryo the kidneys (mesonephroi) increase from 1.3 per cent to 4.8 per cent at 0.83 grams body weight. At about 5 grams body weight the relative weight of the mesonephroi is 3.8 per cent.

At about birth the kidneys form 1.1 per cent of the total body weight. This decreases slowly throughout the series observed and probably throughout life. In a fish weighing 1499 grams the percentage weight of the kidneys is 0.38 per cent.

In the human embryo the mesonephroi form only 0.6 per cent of the body at 11 mm., rapidly decreasing thereafter and practically disappearing at about 30 mm. (Jackson). Lowrey finds the mesonephroi of the pig relatively much larger, reaching a maximum relative size of 12 per cent of the body in the early embryo (15 mm.), decreasing thereafter and practically disappearing at about 125 mm.

*j. Gonads.* (1). Female. In the embryonic dogfish the ovaries are relatively small. In an embryo weighing 0.412 gram the percentage weight of the ovaries is 0.048 per cent. This increases to a maximum of 0.065 per cent in an embryo weighing 2.6 grams. At about 5 grams body weight the percentage averages 0.057 per cent (0.019–0.092).

In the young dogfish the ovaries are relatively much heavier than in the embryo. At about birth the relative weight of the ovaries is 0.4 per cent. At 345 grams body weight this percentage has increased to a maximum of 0.81 per cent. At 1499 grams body weight the percentage is 0.28 per cent. None of the ovaries examined contained large yolk-filled ova.

(2). Male. In the dogfish embryo and at birth the testes are relatively of about the same weight as the ovaries. The percentage increases from about 0.4 per cent at birth to a maximum (for the series) of 1.04 per cent at 133 grams body weight. At 352 grams the percentage is 0.74 per cent.

Kellicott finds the relative weight of the ovaries of the dogfish to rise after birth to a primary maximum of 0.675 per cent in



fish of 400 grams. This percentage decreases to 0.43 per cent in fish of about 1700 grams and thereafter tends to rise to a second maximum. In the male he finds the testes to rise from 0.358 per cent at birth to a primary maximum of 0.775 per cent at about 400 grams. This percentage decreases to 0.60 per cent at 900 grams and then rises throughout life to a final maximum ratio of 1.15 per cent. In the human, Jackson finds the sexual gland relatively larger in the embryo than in the later fetal stages and the testis much larger than the ovary at corresponding stages.

*k. Stomach-intestines.* The following data refer to the empty stomach and intestine.

In twenty-five dogfish embryos, the stomach and intestines form an average of 2.43 per cent (range 1.5 to 4.5 per cent) of the total body weight. At about birth the percentage weight of the stomach and intestines averages 2.9 per cent. After birth this percentage increases, reaching a maximum of 5.5 per cent at 331 grams body weight and thereafter decreasing probably throughout life, being 2.3 per cent at 1499 grams. This post-natal rise is probably directly due to the change from the embryonic to the free living condition—the response to the demand on the digestive system for the digestion of an entirely different diet.

In the human embryo, Jackson finds the stomach and intestines variable. In the newborn, Vierordt estimates that the empty stomach and intestines form 2.1 per cent of the total body weight, and in the adult, 2.06 per cent. In the albino rat, Jackson finds the percentage weight of the empty stomach and intestines in the newborn to be 2.4 per cent, increasing to a maximum of 8 per cent at six weeks and decreasing thereafter to 5 per cent at 1 year. In the pig, Lowrey finds the percentage weight of the stomach and intestines to increase throughout the prenatal period, being (empty) about 3.6 per cent at about full term. In the adult they increase to 4.79 per cent empty. Weleker and Brandt give data on the percentage weight of the adult intestinal tract showing ranges as follows: of fishes, 2.31 per cent to 5.15 per cent; amphibia, 4.32 per cent to 6.05 per cent; reptiles, 4.84 per cent to 5.68 per cent.

## SUMMARY

1. The head attains a maximum relative size of about 40 per cent of the body in the dogfish embryo at 0.09 gram body weight. At birth this has decreased to about 17.5 per cent and continues to fall thereafter, probably throughout life. At 1500 grams body weight, it has dropped to nearly 12 per cent.

2. In general the skin rises through embryonic life to about 11.3 per cent at birth; thereafter it decreases in relative weight to about 7 per cent of the total body weight.

3. At birth the relative weight of the skeleton has risen to about 8.6 per cent. The maximum (about 10 per cent) occurs shortly after birth, and thereafter the relative weight of the skeleton apparently decreases somewhat.

4. The curve of the relative growth of the musculature and skeleton of the embryo is variable and uncertain. At birth the relative weight of the musculature alone is about 45 per cent of the total body; this increases to nearly 63 per cent at a body weight of 1500 grams.

5. In the embryo the viscera decrease in relative size from over 19 per cent of the body at an early period to about 12 per cent at birth. After birth the percentage weight of the viscera attains a maximum of 14.3 per cent (average) at about 350 grams body weight and thereafter decreases to 9.8 per cent at about 1500 grams. The high figures for the early embryos are due chiefly to the relatively large size of the brain.

6. The relative size of the embryonic brain decreases from a maximum of about 15 per cent in the early embryo, at first rapidly and then more slowly to about 1.6 per cent at birth. After birth the relative weight of the brain decreases more slowly, but continues to fall throughout the series and probably throughout life.

7. The spinal cord decreases in relative size in the embryo much like the brain, but it is more variable (probably due to error from difficulty in dissection); in the early embryo it forms 1.76 per cent. At about birth the percentage weight of the spinal cord is about 0.50 per cent. This figure thereafter de-

creases slowly to about 1.7 per cent. In the embryo the spinal cord is usually only about one-tenth as large as the brain, while in the adult it is about one-third as large.

8. The relative weight of the eyeballs of the embryonic dogfish is variable, but in general it decreases from an early maximum of about 9 per cent. At birth it is about 2 per cent and thereafter it decreases slowly. At 1500 grams body weight, the eyeballs form about 0.64 per cent of the body. Throughout postnatal life the eyeballs are as large as the brain and spinal cord combined.

9. In general the heart of the dogfish embryo decreases in relative size from a maximum of about 4 per cent, at first rapidly and then more slowly. At birth it is about 0.15 per cent of the total body weight. After birth it rises to nearly 0.20 per cent and then decreases again. The figure (0.20 per cent) found at about 1500 grams is probably too high, since Kellicott in a larger series finds it much lower.

10. The embryonic pancreas remains at about the same average relative size (0.06–0.07 per cent) for the stages examined. At about birth it forms 0.06 per cent of the body weight, increasing thereafter to a maximum of 0.14 per cent at about 350 grams body weight.

11. In the early embryo the liver rises to a maximum of about 7 per cent of the body. This percentage falls gradually to about 4.8 per cent at birth. Shortly after birth the liver rises and thereafter decreases in relative weight to about 5.9 per cent at 1500 grams.

12. In the embryo the spleen is variable (average 0.049 per cent) but in general it increases in relative weight. At birth the relative weight of the spleen averages about 0.098 per cent of the total body. This figure increases to a maximum of 0.38 per cent at about 350 grams and thereafter decreases. The spleen is exceedingly variable in weight.

13. The relative weight of the rectal gland appears variable in the embryo, but falls from an average of 0.105 per cent to about 0.032 per cent at birth. Shortly after birth the relative weight increases slightly and thereafter decreases.



14. The relative weight of the embryonic kidneys (mesonephroi) increases to a maximum of 4.8 per cent at 1.8 grams body weight, and then slightly decreases. At birth it has fallen to about 1.1 per cent and continues to decrease thereafter to 0.38 per cent at a body weight of 1500 grams.

15. The testes and ovaries of the embryo are relatively of about the same weight. At birth the relative weights of the testes and ovaries is about 0.40 per cent of the body. The testes increase to a maximum of 1 per cent at about 130 grams body weight. The maximum for the ovaries (0.81 per cent) occurs at about 350 grams and the relative weight thereafter falls to 0.28 per cent at about 1500 grams body weight.

16. The percentage weight of the stomach and intestines on the whole increases in the embryo from a minimum of 1.5 per cent to about 2.9 per cent at birth. After birth the stomach and intestines increase to a maximum of 5.5 per cent at about 350 grams body weight and thereafter decrease, probably throughout life.

17. Although many minor differences may be observed, on the whole the course of the relative growth of the various organs and parts in the dogfish is strikingly similar to that which has been observed among the higher vertebrates, including mammals and man.

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TABLE 1  
General table of individual observations (*Mustelus canis*)

NO.	SEX	NET BODY WEIGHT IN GRAMS	LENGTH IN MM.		HEAD	
			Total	Trunk	Net weight	Per cent weight
1	f	1498.800	800.0	385.0	193.000	12.88
2	f	360.472	472.0	215.0	43.792	12.15
3	m	352.481	495.0	225.0	45.426	12.89
4	m	344.081	452.0	210.0	31.266	9.09
5	f	331.512	488.0	218.0	47.746	14.40
6	m	133.439	376.0	168.0	19.771	14.82
7	m	131.444	382.0	173.0	19.416	14.77
8	m	121.270	315.0	145.0	16.223	13.38
9	m	102.295	339.0	151.0	17.628	17.23
10	f	92.790	343.0	151.0	15.393	16.59
11	m	82.110	320.0	143.0	13.881	16.91
12	m	72.350	321.0	143.0	11.641	16.09
13	f	65.620	306.0	141.0	11.364	17.32
14	f	59.160	280.0	125.0	9.578	16.19
15	m	53.243	283.0	124.0	8.941	16.79
16	m	48.835	279.0	130.0	7.705	15.78
17	f	47.544	273.0	125.0	9.793	20.60
18	m	42.075	253.0	115.0	8.138	19.34
19	f	5.260	113.0	54.0	0.709	13.48
20	f	5.033	113.0	55.0	1.071	21.28
21	f	4.982	111.5	53.0	1.139	22.85
22	f	4.814	104.0	49.0	1.178	24.47
23	m	4.772	112.0	52.5	1.113	23.32
24	m	3.871	106.0	49.5	0.962	24.86
25	f	3.834	102.0	48.5	0.875	22.83
26	m	3.826	100.0	46.0	0.779	20.37
27	f	3.797	98.5	47.0	0.965	25.43
28	f	3.652	101.0	48.0	0.916	25.07
29	f	3.516	93.0	46.0	0.695	19.77
30	f	2.606	85.0	43.0	0.507	19.46
31	m	1.744	70.0	36.0	0.301	17.27
32	m	1.139	65.0	32.0	0.321	28.22
33	m	0.826	59.5	28.0	0.238	28.78
34	m	0.746	54.0	26.0	0.189	25.36
35	m	0.525	49.0	24.0	0.166	31.53
36	f	0.412	43.0	21.0	0.126	30.63
37	m	0.370	45.0	20.1	0.126	34.30
38	m	0.334	45.0	20.0	0.099	28.91
39	?	0.313	40.0	21.0	0.095	30.42
40	?	0.251	37.0	19.0	0.072	28.76
41	?	0.187	35.5	18.0	0.059	31.66
42	?	0.104	27.0	16.0	0.039	37.60
43	?	0.092	26.0	15.0	0.037	40.50
44	?	0.061	25.0	14.0	0.023	38.49
45	?	0.051	24.0	12.5	0.011	21.02
46	?	0.045	25.0	13.0	0.016	35.51
47	?	0.008	16.0		0.002	21.43



General table of individual observations (continued)

No.	SEX	SKIN		SKELETON		HEART	
		Net weight in grams	Per cent weight	Net weight in grams	Per cent weight	Net weight in grams	Per cent weight
1	f	109.007	7.27	136.300	9.09	3.003	0.20
2	f	31.041	8.61	32.446	9.00	0.822	0.23
3	m	30.275	8.59	31.186	8.82	0.700	0.20
4	m	22.403	6.51	24.411	7.09	0.503	0.15
5	f	36.211	10.92	40.231	12.14	0.659	0.20
6	m	11.946	8.95	14.000	10.49	0.273	0.20
7	m	15.054	11.45	14.106	10.73	0.236	0.18
8	m	8.985	7.41	8.315	6.85	0.233	0.19
9	m	12.571	12.29	9.059	8.86	0.176	0.17
10	f	13.484	14.53	7.931	8.55	0.194	0.21
11	m	7.246	8.82	6.849	8.34	0.165	0.20
12	m	8.553	11.82	7.636	10.55	0.078	0.11
13	f	8.182	12.47	5.906	9.00	0.124	0.19
14	f	7.375	12.47			0.073	0.12
15	m	5.416	10.17	4.028	7.57	0.058	0.11
16	m	6.443	13.19	2.972	6.09	0.085	0.17
17	f	6.261	13.17	3.498	7.36	0.091	0.19
18	m	3.991	9.49	3.475	8.26	0.076	0.18
19	f	0.368	7.00	0.216	4.10		
20	f	0.430	8.55	0.343	6.82	0.019	0.38
21	f	0.308	6.18			0.007	0.14
22	f	0.364	7.55			0.012	0.26
23	m	0.289	6.06			0.010	0.21
24	m	0.322	8.33			0.007	0.19
25	f	0.278	7.25			0.010	0.26
26	m	0.289	7.56	0.172	4.50	0.006	0.16
27	f	0.274	7.22			0.009	0.23
28	f	0.218	5.98			0.012	0.33
29	f	0.271	7.71			0.015	0.43
30	f	0.227	8.72	0.203	7.80	0.013	0.51
31	m	0.080	4.60			0.012	0.70
32	m	0.068	6.01			0.003	0.25
33	m	0.059	7.08			0.002	0.29
34	m	0.037	4.99			0.003	0.43
35	m	0.030	5.62			0.001	0.25
36	f	0.023	5.63				
37	m	0.025	6.65			0.001	0.19
38	m	0.021	6.27			0.001	0.29
39	?					0.003	1.02
40	?					0.002	0.88
41	?						
42	?					0.0003	0.29
43	?						
44	?					0.0003	0.82
45	?						
46	?					0.002	4.27
47	?					0.0002	2.38

General table of individual observations (continued)

NO.	SEX	BRAIN		SPINAL CORD		EYEBALLS	
		Net weight in grams	Per cent weight	Net weight in grams	Per cent weight	Net weight in grams	Per cent weight
1	f	6.298	0.42	2.497	0.17	9.541	0.64
2	f	2.327	0.65	0.786	0.22	3.331	0.92
3	m	2.059	0.58	0.812	0.23	4.003	1.14
4	m	2.012	0.58	0.766	0.22	3.003	0.87
5	f	3.099	0.93	1.260	0.38	5.260	1.59
6	m	1.666	1.25	0.505	0.38	2.432	1.82
7	m	1.676	1.28	0.577	0.44	2.486	1.89
8	m	1.457	1.20	0.468	0.39	2.153	1.78
9	m	2.300	2.25	0.333	0.33	2.130	2.08
10	f	1.228	1.32	0.348	0.38	1.236	1.33
11	m	1.073	1.18	0.284	0.35	1.781	2.17
12	m	0.946	1.31	0.316	0.44	1.030	1.42
13	f	0.930	1.42	0.239	0.36	1.548	2.36
14	f	0.893	1.51	0.208	0.35	0.955	1.61
15	m	0.746	1.40	0.213	0.40	1.243	2.33
16	m	0.988	2.02	0.279	0.57	0.874	1.79
17	f	0.874	1.84	0.171	0.36	1.316	2.77
18	m	0.786	1.87	0.209	0.50	1.134	2.70
19	f	0.066	1.25			0.116	2.21
20	f	0.109	2.17	0.018	0.36	0.173	3.44
21	f	0.135	2.70	0.014	0.28	0.243	4.87
22	f	0.093	1.94	0.017	0.36	0.186	3.86
23	m	0.132	2.76	0.017	0.36	0.253	5.30
24	m	0.123	3.18	0.010	0.26	0.190	4.91
25	f	0.117	3.04	0.008	0.22	0.200	5.21
26	m	0.074	1.94	0.006	0.16	0.152	3.98
27	f	0.114	3.01	0.009	0.23	0.189	4.98
28	f	0.101	2.78	0.011	0.29	0.185	5.06
29	f	0.062	1.77			0.094	2.68
30	f	0.066	2.54	0.006	0.24	0.075	2.89
31	m	0.039	2.25	0.011	0.64	0.059	3.39
32	m	0.057	5.04	0.004	0.33	0.049	4.31
33	m	0.050	6.06	0.005	0.59	0.045	5.46
34	m	0.045	6.06			0.038	5.12
35	m	0.040	7.63	0.003	0.65	0.027	5.20
36	f	0.036	8.79	0.003	0.78	0.019	4.66
37	m	0.028	7.68	0.003	0.76	0.020	5.30
38	m	0.027	7.85	0.004	1.08	0.017	5.01
39	?	0.017	5.50	0.002	0.70	0.018	5.81
40	?	0.008	3.27			0.018	7.25
41	?	0.020	10.44	0.003	1.77	0.008	4.02
42	?	0.011	10.67			0.006	5.67
43	?	0.007	7.75			0.007	7.42
44	?	0.009	15.13			0.006	9.38
45	?	0.004	8.25				
46	?	0.005	11.24			0.002	3.37
47	?	0.001	15.48			0.0002	2.38

General table of individual observations (continued.)

NO.	SEX	MUSCULATURE		PANCREAS		LIVER	
		Net weight in grams	Per cent weight	Net weight in grams	Per cent weight	Net weight in grams	Per cent weight
1	f	939.600	62.96			88.100	5.88
2	f	198.554	55.08	0.592	0.16	31.923	8.86
3	m	213.814	60.66	0.432	0.12	21.261	6.03
4	m	223.589	64.98	0.444	0.13	29.856	8.68
5	f	171.269	51.66	0.481	0.15	12.964	3.91
6	m	77.146	57.81	0.161	0.12	6.216	4.66
7	m	46.990	35.75	0.191	0.15	4.241	3.23
8	m	33.505	27.63	0.103	0.08	6.413	5.29
9	m	49.327	48.23	0.065	0.06	5.506	5.38
10	f	49.855	53.73	0.058	0.06	5.431	5.85
11	m	40.087	48.82	0.063	0.08	5.239	6.38
12	m	26.436	36.54	0.023	0.03	3.250	4.49
13	f	31.905	48.62	0.041	0.06	3.962	6.04
14	f			0.031	0.05	3.115	5.27
15	m	20.483	38.47			1.666	3.13
16	m	28.360	57.87			2.895	5.93
17	f	22.168	46.42	0.038	0.08	2.513	5.29
18	m			0.032	0.08	1.813	4.31
19	f	1.382	26.25			0.211	4.01
20	f	1.830	36.36	0.003	0.05	0.296	5.89
21	f	2.060*	41.35*			0.224	4.49
22	f	2.445*	50.78*	0.003	0.05	0.237	4.93
23	m	2.211*	46.34*	0.004	0.09	0.183	3.84
24	m	1.636*	42.27*	0.003	0.09	0.161	4.15
25	f	1.774*	46.27*	0.003	0.08	0.151	3.93
26	m	1.890*	49.39*	0.001	0.03	0.203	5.31
27	f	1.346*	35.46*	0.003	0.08	0.163	4.29
28	f	1.991*	54.52*	0.003	0.08	0.181	4.94
29	f			0.002	0.06	0.174	4.95
30	f	0.983*	37.72*	0.001	0.03	0.129	4.93
31	m	0.748*	42.90*	0.001	0.04	0.106	6.09
32	m	0.472*	41.36*	0.001	0.07	0.058	5.05
33	m	0.362*	43.85*	0.001	0.15	0.054	6.49
34	m	0.261*	35.01*	0.0002	0.03	0.034	4.58
35	m	0.208*	39.56*	0.0002	0.04	0.027	5.14
36	f	0.191*	46.41*	0.0001	0.02	0.025	6.12
37	m	0.157*	42.47*	0.0003	0.08	0.019	5.03
38	m	0.152*	44.64*	0.0004	0.12	0.014	4.04
39	?	0.145*	46.39*	0.0002	0.06	0.024	7.73
40	?	0.118*	47.09*	0.0001	0.04	0.016	6.37
41	?	0.091*	48.58*	0.0002	0.11	0.010	5.52
42	?					0.003	2.40
43	?					0.002	1.97
44	?					0.002	2.47
45	?						
46	?					0.001	2.92
47	?						

\* Musculature plus skeleton.



General table of individual observations (continued)

No.	SEX	SPLEEN		STOMACH—INTESTINES		RECTAL GLAND	
		Net weight In grams	Per cent weight	Net weight In grams	Per cent weight	Net weight In grams	Per cent weight
1	f	2.778	0.19	43.800	2.26	0.341	0.02
2	f	1.412	0.39	17.198	4.77	0.162	0.04
3	m	1.249	0.36	13.616	3.86	0.108	0.03
4	m	1.385	0.40	16.467	4.79	0.153	0.04
5	f	1.191	0.36	18.188	5.49		
6	m	0.478	0.36	5.701	1.27	0.073	0.05
7	m	0.386	0.29	5.846	4.45	0.046	0.03
8	m	0.173	0.14	3.985	3.29	0.045	0.04
9	m	0.107	0.10	2.990	2.92	0.036	0.04
10	f	0.103	0.11	2.621	2.82	0.043	0.05
11	m	0.116	0.14	2.190	2.67		
12	m	0.082	0.11	2.034	2.81	0.010	0.01
13	f	0.079	0.12	0.877	1.34	0.027	0.04
14	f	0.053	0.09	1.635	2.76	0.014	0.02
15	m	0.037	0.07	1.297	2.44	0.030	0.06
16	m	0.053	0.11	2.073	4.21	0.025	0.05
17	f	0.056	0.12	1.749	3.66	0.023	0.05
18	m	0.034	0.08	1.468	3.49	0.015	0.04
19	f			0.146	2.78	0.002	0.04
20	f	0.005	0.11	0.091	1.81	0.006	0.12
21	f	0.002	0.04	0.083	1.66	0.005	0.10
22	f	0.002	0.04	0.173	3.60	0.005	0.10
23	m	0.002	0.05	0.215	4.50	0.005	0.11
24	m	0.001	0.03	0.110	2.59	0.004	0.10
25	f	0.001	0.03	0.152	3.96	0.005	0.13
26	m	0.001	0.03	0.083	2.17	0.001	0.03
27	f	0.001	0.03	0.089	2.34	0.005	0.14
28	f	0.001	0.03	0.086	2.36	0.005	0.14
29	f	0.001	0.03	0.065	1.85	0.002	0.06
30	f	0.002	0.08	0.056	2.16	0.001	0.05
31	m	0.001	0.06	0.041	2.36	0.001	0.04
32	m	0.001	0.07	0.028	2.42	0.002	0.13
33	m	0.0004	0.05	0.015	1.85	0.001	0.13
34	m	0.001	0.08	0.016	2.17	0.001	0.09
35	m	0.0002	0.04	0.009	1.68	0.001	0.15
36	f	0.0002	0.05	0.006	1.50	0.0002	0.05
37	m	0.0002	0.05	0.008	2.22	0.0002	0.05
38	m	0.0002	0.06	0.007	1.93	0.0001	0.03
39	?	0.0002	0.06	0.006	1.98	0.001	0.22
40	?	0.0001	0.04	0.005	2.07	0.001	0.24
41	?	0.0001	0.05				
42	?			0.004	3.75		
43	?			0.003	2.73		
44	?			0.001	2.30	0.0001	0.16
45	?						
46	?						
47	?						

General table of individual observations (continued)

NO.	SEX	KIDNEYS		GONADS	
		Net weight in grams	Per cent weight	Net weight in grams	Per cent weight
1	f	5.661	0.38	4.228	0.28
2	f	2.901	0.80	3.096	0.86
3	m	2.556	0.73	2.621	0.74
4	m	2.556	0.74	2.046	0.59
5	f	2.702	0.82	2.559	0.77
6	m	1.188	0.89	1.389	1.04
7	m	1.094	0.83	0.696	0.53
8	m	0.983	0.80	0.503	0.41
9	m	0.996	0.97	0.524	0.51
10	f	0.888	0.96	0.294	0.32
11	m	0.714	0.87	0.234	0.28
12	m	0.560	0.77	0.363	0.50
13	f	0.981	1.49	0.274	0.42
14	f	0.593	1.00	0.197	0.33
15	m	0.440	0.83	0.128	0.24
16	m	0.693	1.42	0.224	0.46
17	f	0.773	1.61	0.201	0.42
18	m	0.447	1.07	0.151	0.36
19	f	0.157	2.97	0.001	0.02
20	f	0.167	3.32	0.003	0.06
21	f	0.243	4.88	0.005	0.09
22	f	0.200	4.16	0.003	0.05
23	m	0.181	3.80	0.002	0.05
24	m	0.162	4.19	0.003	0.08
25	f	0.136	3.56	0.002	0.05
26	m	0.119	3.12	0.002	0.05
27	f	0.142	3.74	0.002	0.04
28	f	0.135	3.70	0.002	0.06
29	f	0.095	2.71	0.002	0.06
30	f	0.091	3.50	0.002	0.07
31	m	0.064	3.68	0.001	0.07
32	m	0.055	4.80	0.0004	0.04
33	m	0.038	4.59	0.001	0.08
34	m	0.014	1.90	0.0002	0.03
35	m	0.013	2.49	0.0004	0.08
36	f	0.009	2.23	0.0002	0.05
37	m	0.012	3.11	0.0002	0.05
38	m	0.005	1.44	0.0002	0.06
39	?	0.010	3.26	0.0004	0.13
40	?	0.004	1.67	0.0004	0.16
41	?	0.004	1.92	0.0001	0.05
42	?				
43	?				
44	?	0.001	1.81		
45	?				
46	?	0.001	1.35		
47	?				

## PHILADELPHIA ACADEMY OF SURGERY

The Samuel D. Gross Prize, Fifteen Hundred Dollars. Essays will be received in competition for the prize until January 1st, 1915.

The conditions annexed by the testator are that the prize "shall be awarded every five years to the writer of the best original essay, not exceeding one hundred and fifty printed pages, octavo, in length, illustrative of some subject in Surgical Pathology or Surgical Practice, founded upon original investigations, the candidates for the prize to be American citizens."

It is expressly stipulated that the competitor who receives the prize, shall publish his essay in book form, and that he shall deposit one copy of the work in the Samuel D. Gross Library of the Philadelphia Academy of Surgery, and that on the title page, it shall be stated that to the essay was awarded the Samuel D. Gross Prize of the Philadelphia Academy of Surgery.

The essays, which must be written by a single author in the English language, should be sent to the "Trustees of the Samuel D. Gross Prize of the Philadelphia Academy of Surgery, care of the College of Physicians, 19 S. 22nd St., Philadelphia," on or before January 1, 1915.

Each essay must be typewritten, distinguished by a motto, and accompanied by a sealed envelope bearing the same motto, containing the name and address of the writer. No envelope will be opened except that which accompanies the successful essay.

The Committee will return the unsuccessful essays if reclaimed by their respective writers, or their agents, within one year.

The Committee reserves the right to make no award if the essays submitted are not considered worthy of the prize.

WILLIAM J. TAYLOR, M.D.

RICHARD H. HARTE, M.D.

JOHN H. GIBBON, M.D.,

*Trustees.*

*Philadelphia, 1914.*



# THE ERUPTION AND DECAY OF THE PERMANENT TEETH

## PRELIMINARY REPORT

ROBERT BENNETT BEAN

*From the Anatomical Laboratory, Tulane University*

Data: 2221 school children

630 Filipino male (five to thirty years)  
776 146 Filipino female (five to thirty years)

322 German male  
628 306 German female

407 American male (five to eighteen years)  
817 410 American female (five to eighteen years)  
2221 Total

## GENERAL RESULTS

### *Eruption of the teeth*

The Filipinos are from one to four years earlier than the Germans and Americans in the eruption of the permanent teeth, and the Americans are slightly earlier than the Germans.

The females are more precocious than the males in the three groups, but this difference is very slight among the Filipinos, and a little less among the Germans than among the Americans.

The Filipinos are more homogeneous sexually (there is less difference between the sexes) than the Americans, who are more heterogeneous than the Germans.

The lower teeth erupt before the upper, except that the upper premolars erupt before the lower. The permanent teeth erupt

at three periods, about the ages of 7, 10 and 18 years, in connection with the eruption of the three sets of molars, and the first two periods alternate with periods of rapid growth in stature.

### *Individual teeth*

The teeth erupt in the following order:

- |                           |                             |
|---------------------------|-----------------------------|
| 1. Lower first molars     | 9. Lower median premolars   |
| 2. Lower median incisors  | 10. Upper lateral premolars |
| 3. Upper first molars     | 11. Upper canines           |
| 4. Upper median incisors  | 12. Lower lateral premolars |
| 5. Lower lateral incisors | 13. Lower second molars     |
| 6. Upper lateral incisors | 14. Upper second molars     |
| 7. Upper median premolars | 15. Lower third molars      |
| 8. Lower canines          | 16. Upper third molars      |

This order is followed by the Germans and Americans, and also by the Filipinos except that among the Filipinos the canines erupt earlier than the premolars and upper lateral incisors, and the canines erupt from two to four years earlier in the Filipinos than in the Germans and Americans.

### *The law of alternation in development*

A law of alternation in development has been deduced, based upon the alternation of periods of acceleration and retardation in the growth of the long bones (stature), upon the periods of acceleration and retardation in the development of the permanent teeth, as well as from a general knowledge of development, especially from the researches of Donaldson, Jackson and others. This law may be formulated somewhat as follows:

*There are one or more periods of acceleration alternating with periods of retardation in the development of the structures of the body. The periods of acceleration in the development of one structure are synchronous with the periods of retardation in the development of another.*

The various structural parts, or organs, of the body do not develop synchronously, nor with equal rapidity during the same periods of time, but first one then another develops. Thus the

period of the first six months after birth is one of rapid growth in length which is followed by the eruption of the temporary teeth, all of which are through the gums by the end of the third year, after which there is a period of rest. Following this there is another period of rapid growth in length (stature), subsequent to which the permanent teeth begin to erupt, after which the growth of the body is again accelerated, to be followed by a second rapid eruption of the permanent teeth, and then another rapid growth of the body which is succeeded by puberty.

The development of the organs in the embryo and the fetus, as well as after birth may be given to illustrate the law of alternation. The early development of the heart precedes that of the lungs, the late development of the liver precedes that of the stomach and intestine, and the development of the brain and head precedes that of the trunk and extremities.

The law is not only applicable to normal development but also seems to apply to abnormal development through a process of compensation. If one structure is unusually precocious in the periods of acceleration in development, its complementary structure will be backward in the periods of acceleration, and vice versa. Thus the upper canines are precocious in the Filipino boys, and the upper lateral incisors are backward, and the upper lateral incisors are precocious in the Filipino girls and the upper canines are backward. Other examples could be cited but these suffice to illustrate the law.

#### *Decay of the teeth*

The temporary teeth of the Americans are worse than those of the Filipinos which are worse than those of the Germans. The permanent teeth of the Americans are worse than those of the Germans which are worse than those of the Filipinos. The girls have worse teeth than the boys in all the groups.



*Morphologic form and teeth*

Those individuals with long faces, heads and noses, and large occipital circumferences of the head have worse teeth than those individuals with broad heads, faces and noses and large parietal circumferences of the head, and the teeth of the former develop earlier than the teeth of the latter.

The long head-face-nose forms with the large occipital region of the head have been called Hyper-onto-morphs by me, and the broad head-face-nose forms with the large parietal region of the head have been called Hypo-onto-morphs.

The relative number of Hyper-onto-morphs is greatest among the Americans, least among the Filipinos, and nearly as great among the Germans as among the Americans. Hypo-morphism decreases with age, and Hyper-morphism increases, so that whereas among the Filipinos there are 15.2 Hypos to 1 Hyper between the ages of five and sixteen, there are only 3.8 Hypos to 1 Hyper from 16 to 30 years of age. Hypo-morphism is a condition of less maturity than Hyper-morphism. Apparently the Filipinos mature more slowly than the Americans and Germans in morphologic form, although they mature earlier in stature and in the eruption of their permanent teeth, which, again, may be only another expression of compensation in the law of alternation in development.

## OSTEOLOGY REDIVIVUS: A CRITICISM

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It has been a time-honored custom to introduce the student of medicine to anatomy by a course in osteology. Since this course was, as a rule, preliminary to dissection, the student obtained his first impressions of human anatomy from it. While not underestimating the influence of the individual teacher in the present, the personal element in the past, no doubt, played a far more important part in the presentation of this or of any other laboratory subject, because the laboratory equipment was generally inadequate or more often lacking altogether. Not an extensive equipment was needed for osteology, to be sure, but as long as the student had to go to a laboratory to study bones literally chained to the desk or where he might look at a skeleton through glass doors, none but the most persevering and resolute could be expected to take more than a compulsory or perfunctory interest in the subject, unless, as was fortunately often the case, stimulated by the personality of an inspiring and enthusiastic teacher.

The exact nature of the work usually given is known better to others than to myself, but generally the student merely studied a book or manual containing a bare, even if a detailed, description of individual bones; and the instructor failed to present the much broader and far more interesting aspects and relations of the subject. Under such circumstances bones were dry indeed and it is not at all surprising that these conditions originated and perpetuated the oft-used phrase "dry as bones."

It is to be regretted that the present teaching of osteology has not been wholly liberated from the onus of this phrase or from the burden of such conditions. If the study of osteology must be reduced to the mere memorizing and recognition of the external features of bones, then there is no escape from making it more

than a mere task of memory—a sort of mental gymnastics—the burden of which is not lightened appreciably by lectures and recitations in which the lecturer recites a textbook description on one day and the student in turn recites it the next. The use of cues, or of mnemonic crutches on the part of the student, or of summaries on the part of the lecturer, stating that there are “eleven points of interest on the inferior surface of the temporal bone” or that there are so many angles or processes on this or that bone, can really not mitigate matters much even if the applicant for licensure passes his examination with a score of one hundred. The latter is an accomplishment wholly within the power of any highschool boy with a good memory, who has never been in an anatomical laboratory. Neither can drawing bones or modelling them in clay, however valuable as adjuncts, do much to relieve such drudgery. Not that the reproduction in clay of the main features of bones is not worth while and may not impress the form of bones more lastingly upon the mind, but until sufficient control over the mechanics of modelling has been acquired the student’s attention is occupied largely with the details of a process rather than with the real end to be attained. Moreover, most students manifestly cannot obtain sufficient mastery over the art of modelling in the time at their disposal, to be able to reproduce the relief of bones with the necessary detail. Hence only the most obvious things are emphasized while the less obvious and the less evident but often the more significant features, are likely to be overlooked. Besides, the end in view manifestly is not the production of a fine model but the acquisition of knowledge and an acquaintance with the subject. It is the same with drawing. Both must ever remain mere means to an end. One or both may be indispensable for some or even for the majority of students but in any case they can be of value only by stimulating and assisting in training powers of observation and by increasing interest in and knowledge of a subject. To be sure, the employment of these aids may also enrich the student’s armamentarium and make him a better craftsman, but the better-trained, the maturer and the more capable he is, the less will he rely upon such accessories for obtaining a grasp of any subject, however difficult.



Hence it seems to me that the true attitude on the part of both teacher and student, to these or any other aids, however valuable, during years devoted primarily to discipline, should be an entirely volitional and not an obligatory one, for this is the time when the mature student should be thrown upon his own responsibility and his creative powers given free play. A plastic and capable mind, I presume, never does end its days of self-training, but any mature student who prefers to dispense with all prescribed aids, or with his teacher for that matter, should be permitted to do so. Training of the mind must naturally continue but personal guidance should become less and less necessary. This shifts the burden from the instructor's to the student's shoulders and holds women and men preparing for a serious profession, responsible for the consequences of their own acts. And surely young women and young men who have had three or four or more years of college training after passing the highschool, cannot be subjected to pedagogical absolutism without inviting and engendering antagonism, or very seriously thwarting initiative and destroying their interest in a subject. Hence it seems to me that entire freedom — and responsibility should characterize the work of mature students in medical as in graduate work. Freedom on the part of the student to adopt whatever means he prefers in the attainment of a definite or of a given end, is absolutely essential and the attainment of that end should be made the chief rule by which he is measured. But freedom on the part of the instructor from pedagogical routine of all sorts and freedom from responsibility for failure on the part of every student to reach a certain specified end at a certain definite time is just as essential. For however disastrous *laissez faire* may have been and still is, in the industrial world or in the kindergarten, it is a *sine quo non* for the spontaneous intellectual development of women and men. We may compel scholarship in boys and girls but the intellects and capacities of women and men who have chosen their life's work, I take it, can be truly developed only where supervision is reduced to a minimum and where in the familiar words of Ulrich von Hutten, "der Wind der Freiheit weht."

If graduate work can prosper only where and when students are freed from pestiferous intermeddling and allowed or made to assume responsibility for themselves, then neither can medical education be at its best in a highschool atmosphere. And surely in the profession of medicine, we are dealing with women and men and not with girls and boys. It is true that some educated Englishmen still speak of boys of sixteen who are attending this or that preparatory school, beginning the study of medicine, but that is fast becoming impossible in this country. Girls and boys cannot study medicine to advantage nor prosper in an atmosphere congenial to, and indispensable for the best development of maturer minds. I am well aware that the granting and the assumption of freedom implies a risk; perhaps too great a risk for some medical students, but that is unavoidable. It is a well-known axiom that we cannot have freedom without risks and that if we are unwilling to take the risk we cannot have freedom or the golden fruit which it alone matures.

If the student who is beginning the study of osteology is given free access to abundant material and enabled to see that the many details which he must acquire are only pebbles on a path which he must travel before the enjoyment of the broader view is possible, his attitude will soon change. He will not simply grind and memorize but think and inquire, or at least grind, think and inquire. Lectures in osteology, to be sure, need not be confined to or even include a rehearsal of the detailed descriptions, however excellent, found in our textbooks of human anatomy. For there certainly are many aspects of osteology, a proper discussion of which will not only invite but command the attention of students. I refer to the many interesting questions connected with the development, the growth, form, structure, nutrition, regeneration, transplantation and adaptation of bones and the significance of variations and deformities, not to mention the long and attractive vista offered by comparative anatomy, physical anthropology, or even archeology. Here are a multitude of things of sufficient value to challenge the attention and hold the interest of the maturest students. Surely the timely discussion of these matters can easily convert the dry and dead into a living bone, and a grind

into a not altogether unpleasant task. Moreover, if the attention of the student were directed more to the living than to the dead bone much would be gained by this alone, for it is after all the secrets and the nature of the living that we are trying to learn from a study of the dead.

The plea is not for separate courses in osteology. As far as they are concerned, there is no more reason for them than for separate courses on any other system of organs. Nor is there much more reason for modelling bones than hearts, livers, kidneys, the brain or other viscera. It matters little, to be sure, whether the facts in question are presented in a separate course preceding the dissection, or, as is more desirable perhaps, parallel with them and correlated as far as possible with the rest of the subject of anatomy. However, the mere inclusion or omission of a special course on osteology among the courses of anatomy in any institution, no matter how high its standing, does not necessarily imply that the subject is or is not presented as is desirable. But the general considerations on any system of organs are surely of sufficient interest and importance to warrant their presentation in a more or less formal way and separate form. That, however, is purely a matter of individual preferences and of no special moment. The value lies, not, to be sure, in this or in that method of presentation, but in the value of the facts themselves.

The oldfashioned, separate, preliminary courses in osteology derived their main use from the fact that the illustrations and descriptions in the texts in use by the students, were often inadequate and that students did not have sufficiently free access to the bones themselves. But these things need no longer be so and it is seriously to be doubted whether many of the oldfashioned lectures were an improvement on the textbook descriptions. Many of those giving them were not trained or interested in anatomy, as a rule, beyond a knowledge of the elementary facts. They were, after all, professed devotees whose main allegiance was given to the practice of medicine and not to anatomy.

It is the wider and the larger outlook that is needed in many places even now. The student must learn to look behind, not beyond, the facts, for loose generalizations are worse than none.



But unfortunately the subject-matter of osteology has been restricted until it is devoid of interest and the study of it has become a repulsive task. But that is not the fault of the subject. Under such conditions it is wise that special courses in it be omitted. We have an incomparable legacy which deserves the best presentation that time and the ability of the instructor permit. There is absolutely no reason why the study of osteology should be limited to the minimum of what is *supposed* to be needed by the future practitioner. That is indeterminable. To be sure, if anatomists neglect to see to it that students of medicine have the knowledge of anatomy indispensable in practice, then others must and will see to it. But unfortunately a limitation of the subject to what is supposedly needed in practice has very largely been the custom not only in the teaching of osteology, but in the presentation of the entire subject of human anatomy. Any subject in the whole range of the medical or in other sciences would be sterile if so reduced and taught. There is, of course, a certain minimum of information which every medical student must have to be a safe practitioner of medicine but the presentation of the subject should not therefore be so limited. Not that anatomists cherish the foolish desire to make disciples out of most medical students. Some of them are and may become, good dissectors during their years of training, but an anatomist verily must ever be the product of longer seasons, of riper years and broader perspectives. This holds, I take it, even if we do not entertain for anatomists the exalted conception of Paracelsus regarding physicians, viz., that they are not made in highschools but are *born* and made by the Lord himself! While, then, few students can or will ever become anatomists, I see no reason why the subject should not be presented as though all *would become* anatomists. Nor is it therefore necessary to overlook or to regret the fact that we are training future practitioners. The frank recognition of this fact makes the adoption of the broader view all the more necessary and important to these individuals whose future life's work will be barren and a drudgery without it. There may not be much untilled ground in osteology but the student's conception of and interest in a subject is but rarely determined by such consider-

ations. The latter applies only to the few—the very few—and these will not confine their attention to osteology alone but will claim the whole field of anatomy in which the harvest has already been a rich one even if full many a sheaf lies yet ungarnered.

Unfortunately, most of our textbooks have omitted what might be called introductory chapters on general anatomy, in which the fundamental facts regarding the various systems are presented. Some of them contain a few sentences or paragraphs on the general aspects of the various systems but no connected account is given. For a connected account of so simple a thing as the skin or the superficial fascia, for example, the student must search the literature or special monographs on the subject. It seems to me that there is every reason why the student should be assisted in welding the discouraging number of details on each subject into a consistent whole, largely through the medium of the many interesting general considerations or special relations. The wealth of these is as great as the need for their presentation is grievous. That is also true of the question of muscular movements, to a knowledge of which practicing neurologists have added so much in recent years by their study of injuries and paralyses. Nevertheless, our textbooks of anatomy continue merely to tabulate muscular activity as though each muscle acted as independently as the enclosure about its name in the customary table would seem to suggest. It is no wonder that the student thinks of independent, isolated motions rather than that of associated common movements, of facts instead of their significance. In this case a subject of surpassing interest and of great practical value is reduced to the level of a table which however useful, gives not the least intimation of the great complexity of muscular movements. Surely the work of Bell, Duchenne, Hunter, Winslow and others, deserves better than this, and while I fully realize that the days of Haller and Johannes Müller are long passed, yet here is an opportunity for reform. No one pretends "to make all knowledge his province" and I gladly leave to physiologists what is theirs, but there surely is sufficient cause for Roux's criticism that "Die menschlichen Anatomen, welche Funktionen der Gelenke und Muskeln and der *Leiche* studieren und nach diesen Befunden lehren, sind

in diesem Falle immer schon reine Analytiker gewesen. Denn sie erforschen die *möglichen* Funktionen der *einzelnen* Gelenke und der einzelnen Muskeln. Manche aber erachten dann diese Ergebnisse ohne weitere Prüfung als für die Lebenstätigkeit gültig, obschon in der Wirklichkeit des Lebens diese *einzelnen* Funktionen manchmal gar nicht vorkommen." There are those who have long believed in the presentation of the whole subject from a functional standpoint, as counselled recently by a distinguished investigator. To ask constantly how a structure is built without stopping for a moment to inquire why is it built thus or so, would hardly seem possible for intelligent beings and would reduce the study of anatomy to mere memorizing, for as has been well said: "Und Anatomie allein getrieben, ohne Bezugnahme auf die Funktion, scheint manchmal eine recht sterile Wissenschaft." There is, to be sure, a limit to the capacity and knowledge of every one of us, yet the embryological, comparative anatomical, and anthropological aspects must not be forgotten, for these things alone can re-invigorate what has been reduced to the dead level of detached facts, and make both teaching and learning interesting. For these twin brothers, of course, must ever travel hand in hand even if one or the other, by birth or station or both, is the favored partner of the two.

So instead of banishing osteology I would revamp and rejuvenate it. I would return to the custom of our great exemplars. It is true that the whole subject of human anatomy has some more or less unavoidably forbidding aspects, yet these are purely incidental and in spite of them human anatomy is of sufficient interest to command the attention of other than medical students. Such an achievement is worth while and will do much, I take it, to free anatomy from the onus cast upon it in the past. It has carried the burden of the old dissecting-room atmosphere, old-fashioned lectures and the things that were associated with it and with them, altogether too long, and what a burden it was and is! External need no longer burden us, for a practically odorless dissecting-room need no longer remain a remote possibility, but may become a present accomplishment.



It is not a question of making anatomy more interesting. Human anatomy *is* interesting and those who do not think so should not profess to be its devotees, nor should they who consider teaching a drudgery be permitted to teach. These words are not meant as a dictum but as a statement of fact which needs emphasis and deserves more general acceptance even in these days. I have no ex cathedra opinions to offer on any subject but I hope that this gentle counterblast will not idly "vex the air" of a silent night and like the cuckoo in June "be heard but not regarded."

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

**DEVELOPMENT AND ANATOMY OF THE NASAL ACCESSORY SINUSES IN MAN.** Based on 290 lateral nasal walls, showing various stages and types of development from the sixtieth day of fetal life to advanced maturity; by Warren B. Davis, M.D., Corinna Borden Keen Research Fellow, Jefferson Medical College, Philadelphia. Octavo, 172 pages with 57 original illustrations by Dorothy Peters. Philadelphia and London: W. B. Saunders Company, 1914. Cloth, \$3.50 net.

Foreword. The literature concerning the embryology, later development, and adult anatomy of the nasal accessory sinuses, is rather abundant, yet the differences in the views expressed—especially concerning the extent of development during the years of childhood—seemed sufficiently great to warrant further study.

The author therefore has collected and carefully studied this series of preparations of the accessory sinus areas—which series covers the various stages of development from the sixtieth day of intrauterine life to advanced maturity—hoping to supply information regarding some few points with which we have been imperfectly acquainted, on account of the scarcity of specimens showing the conditions present during the years of childhood.

Deductions drawn from a few observations are open to fallacy, owing to variations in the extent and type of development as found in different specimens of approximately the same age. In this series an endeavor has been made to obtain a sufficient number of cases showing the various stages of development to make the general averages of practical value.

The bodies of children between the ages of two and sixteen years being seldom obtainable in the dissecting rooms of European institutions as well as in America, it was necessary, in order to complete such a series, to develop a technic by which the accessory sinus areas could be removed en masse at the time of postmortem examinations, and still allow reconstruction of the face without marked disfigurement. Ninety-six of the cases in this series were thus obtained from the post-mortem room of the Friedrichshain Krankenhaus, Berlin.

The material for the other post-natal preparations was furnished by the Daniel Baugh Institute of Anatomy of Philadelphia.

**CARCINOMA OF THE THYROID IN THE SALMONOID FISHES.** An investigation and experimental study conducted jointly by the Gratiwick Laboratory of the State Institute for the Study of Malignant Diseases, Buffalo, N. Y., and the United States Bureau of Fisheries, Harvey R. Gaylord, M.D., Director, State Institute for the Study of Malignant Disease, Buffalo, N. Y., and Millard C. Marsh, Biologist, State Institute for the Study of Malignant Disease, formerly Scientific Assistant, United States Bureau of Fisheries, with the collaboration of Frederick C. Busch, M.D., Internist, and Burton T. Simpson, M.D., Pathologist, State Institute for the Study of Malignant Disease; 162 pages and 127 figures (9 in colors); from Bulletin of The Bureau of Fisheries, Volume 32, 1912, Document No. 790, issued April 22, 1914, Government Printing Office, Washington, D. C.

## A TRANSITIONAL TYPE OF CERVICAL RIB

BARTON G. DUPRE

### WITH A COMMENTARY

T. WINGATE TODD

*From the Anatomical Laboratory, Western Reserve University, Cleveland, Ohio*

FOUR FIGURES

The case of cervical rib about to be described, occurred in the dissecting room of this School, some few months past. Although much literature is available on the subject of cervical rib, we have thought it advisable to give as full a description as possible, of the present instance, because it exhibits certain interesting points which are not clearly dealt with in the majority of previous records. Some facts, however, I have been unable to ascertain, owing to dissection having been performed on the subject previous to the discovery of the condition.

#### DESCRIPTION OF SPECIMEN

Subject VI: Male, white; age, 45 years. Clinical records give no indication of right- or left-handedness.

#### CONDITION OF BONES

1. Occipital bone and condyles, normal.
2. Vertebral column: No scoliosis or other curvature. Atlas vertebra, normal; axis vertebra, normal; 3rd and 4th vertebrae; normal vertebrae, with bifid spines. 5th and 6th vertebrae, spine single; single foramen transversarium on both sides. 7th vertebra (fig. 1), spine single; this is the first rib-bearing vertebra. On each side the foramen transversarium is completed by the rudimentary rib. Left transverse process is directed horizontally outward; the right is shorter and inclined somewhat



caudally. 8th to 19th vertebrae (inclusive), normal rib-bearing vertebrae. Transverse processes of 8th vertebra directed upward and outward. 20th to 24th vertebrae (inclusive), normal lumbar vertebrae. 25th vertebra, normal 1st sacral vertebra. Sacrum, normal; coccyx, fused to sacrum.

The vertebral column may be arranged thus: Atlas; axis; four non-rib-bearing vertebrae; thirteen rib-bearing-vertebrae; five non-rib-bearing vertebrae; sacrum.

3. The sternum presented no irregular features. Episternal bones were absent. The 2d costal cartilages articulated with the junction of manubrium and body. The first seven costal cartilages on each side articulated with the sternum directly. A lateral articulation was present between the 5th, 6th and 7th costal cartilages. The costal cartilages of the 8th and 9th ribs on both sides were connected indirectly with the sternum through their articulation with the costal cartilage immediately preceding. The 10th, 11th and 12th dorsal ribs presented free ventral extremities.

#### *Rudimentary ribs (fig. 1)*

*Right side.* The right rudimentary rib was fused at its head and tubercle with the body and transverse process of the seventh vertebra. It represented a free downwardly projecting pointed outer extremity. No fibrous band was present to connect the free extremity with the first complete rib or the sternum. The length of the rib from head to free extremity was 3.5 cm. The upper aspect presented a well marked groove for the lodgement of the 7th nerve. This groove curved round the ventral side of the rib so that the nerve in the dissection was found emerging apparently *below* the rib. Lateral to the groove the upper aspect was rough for the attachment of the large intertransverse muscle which ran from the 6th to the 7th transverse process.

The tip, lower aspect and dorsal border gave origin to the small scalenus medius muscle.

*Left side.* On the left side the rudimentary rib was longer than on the right. It presented head and tubercle, which, however, were not fused with the vertebra as on the right side but articu-

lated with body and transverse process by means of rudimentary joints. As on the right side the rib presented a free pointed extremity projecting downward and outward and without fibrous connection to sternum or first complete rib. The length of the rib measured along the central line of the upper aspect from head to free extremity was 5 cm. The upper aspect of neck and shaft displayed a groove for the lodgement of the 7th nerve. There was also a tiny transverse groove for the passage of the deep cervical



Fig. 1 Seventh cervical vertebra with rudimentary ribs; first dorsal ribs. One-half natural size. The rudimentary ribs lodged the seventh nerve on each side in the grooves marked *N*. On the left side the deep cervical artery crossed the rudimentary rib in the groove, *A*. The first complete (dorsal) ribs showed a groove for the subclavian vein, *V*, but the sulcus subclaviae was absent.

artery. Lateral to the nerve groove and on both sides of the arterial channel the upper aspect was rough for the attachment of the intertransverse muscle (cf. right rib). As on the right side the free extremity, under surface and dorsal border provided attachment for the small scalenus medius muscle.

*First complete ribs* (fig. 1). These when placed on the table lay quite flat. They were therefore not of the 'rocker' pattern (2). They presented the usual characters of the first rib and displayed grooves for the subclavian vein: the right groove being somewhat better marked than the left. On both sides the sulcus subclaviae was absent. The right rib was 13 cm. in length from head to an-

terior extremity measured along the center of the upper surface of the shaft. The left was 14 cm. in length. Both articulated with the sternum by means of an intermediate costal cartilage. The angle subtended by each rib from the horizontal was 45 degrees.

The other ribs presented no irregularities, but one or two facts may be mentioned concerning the lower members of the series on each side. The 10th, 11th and 12th complete ribs were of the 'floating' variety.

*Eleventh complete ribs.* Typical appearance; length on each side 19 cm.

*Last ribs.* These, the 12th complete pair, articulated with the body of the 19th vertebra and belonged to the short variety (Pansch) (3). They presented a head but no tubercle, and had a free terminal extremity. Unlike the 11th pair, which were very obliquely placed, this pair was directed almost horizontally. The length of the right rib was 6 cm.; that of the left, 5.5 cm.

#### *Scalene group of muscles (figs. 2 and 3)*

It is to be understood that these muscles were similar on both sides except where differences are indicated.

*Scalenus anterior.* Origin by tendinous slips from the anterior tubercles of the transverse processes of the 5th and 6th cervical vertebrae. Normal insertion by tendinous and fleshy slips into the inner border of the first complete rib.

*Scalenus medius.* Origin by mixed fleshy and tendinous fibers from the outer border and adjacent part of the lower surface and tip of the rudimentary rib. Insertion to the upper aspect of the dorsal three-fifths of the first complete rib. On both sides the superficial fibers of this muscle were continuous with the inter-transversalis muscle which had its insertion into the upper surface of the rudimentary rib.

*Scalenus posterior.* Origin by tendinous slips from the posterior tubercles of the transverse processes of the 2nd, 3rd, 4th, 5th and 6th cervical vertebrae. The insertion of the bulk of the muscle was by tendinous fibers into the outer surface of the second



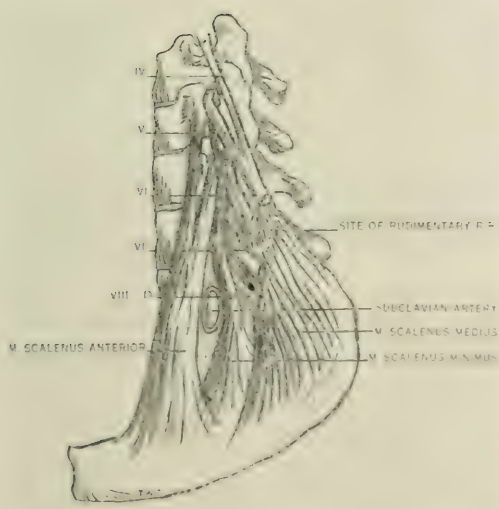
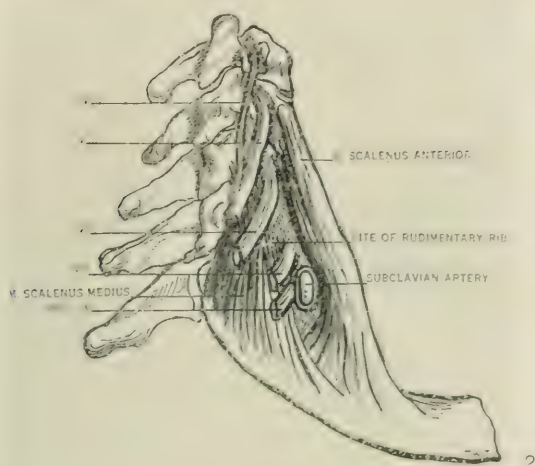


Fig. 2 Dissection of scalene muscles on right side. One-half natural size. The relation of the muscles to the subclavian artery and the nerve-trunk is shown. M. scalenus posterior is not included in the drawing; the nerve-trunks are indicated by numerals.

Fig. 3 Dissection of scalene muscles on the left side. One-half natural size. The relation of the subclavian artery and nerve-trunks to the muscles is illustrated. M. scalenus posterior not shown; nerve-trunks indicated by numerals.

complete rib. Some of the deeper fibers were inserted into the rudimentary and first complete ribs.

*Scalenus minimus.* This muscle was present on the left side only. Its origin was by tendinous fibers from the anterior tubercles of the transverse processes of the 5th and 6th cervical vertebrae. Its insertion was into the inner border of the first complete rib immediately dorsal to and partially continuous with the insertion of the scalenus anterior.

*The intercostal group of muscles.* These showed no direct continuity between their fibers and those of the scalene muscles.

#### *The arteries*

The arteries were arranged as follows: The right subclavian artery arched outward in its course, there being a marked isthmus where it passed ventral to the free extremity of the rudimentary rib and deep to the M. scalenus anterior (fig. 4). This constriction was not due, however, to any effect of the rib or muscle. The isthmus of the artery measured 9 mm. in length of which 4 mm. lay distal to the outer border of the scalenus anterior. Its branches were all given off from the first part, namely, vertebral, thyreo-cervical, internal mammary and costo-cervical.

The deep cervical artery arose from the costo-cervical vessel and passed backward between the rudimentary rib and the first complete rib.

The left subclavian artery also arched outward in its course and presented an isthmus at the summit of the curve just beyond where the vessel passed between the scalenus anterior and scalenus minimus muscles. The narrowing of the vessel was more marked than on the right side. The branches of the left artery included the vertebral, thyreo-cervical and internal mammary vessels from the first part and the costo-cervical from the second part. The deep cervical artery, a branch of the costo-cervical trunk, passed upward and outward deep to the scalenus minimus muscle, then between the 6th and 7th nerve-trunks of the brachial plexus and over the upper surface of the rudimentary rib where it presented an 'isthmus' and occupied a groove on the bone.

Thence it traversed the substance of the intertransverse muscle deep to the scalenus posterior after which it gave off two terminal downwardly directed branches. In its course the vessel supplied twigs to the scalenus minimus, intertransverse and scalenus posterior muscles.

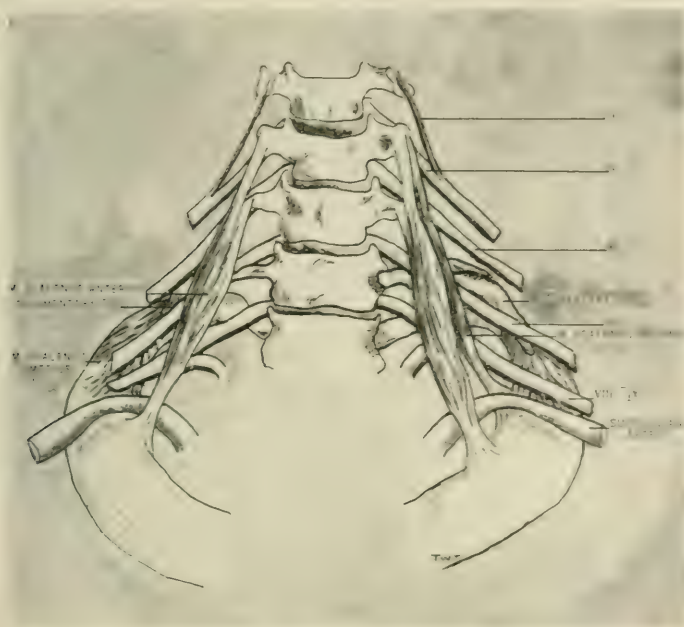


Fig. 4 Drawing to show relation of nerve-trunks to cervical ribs. One-half natural size. The subclavian arteries have been displaced downward to give a better view of the lower nerve-trunks.

*Measurements of subclavian arteries*

	mm.
Length of isthmus on left side.....	12.0
Length of isthmus on right side.....	9.0
Diameter of isthmus on left side....	3.5
Diameter of isthmus on right side.....	5.0
Diameter of left artery above outer border of first complete rib	7.0
Diameter of right artery above outer border of first complete rib..	5.5

No flattening of the arteries was observed. The cirroid form described by Mr. Todd (1) was not well marked in this specimen. The wall of the arteries was not histologically examined.



*The pleura*

The pleura was in direct contact with, though not adherent to, the rudimentary rib on both sides.

*The nerves*

On investigation of the nerves the following results were obtained:

The brachial plexus on the right side was constituted by the 5th, 6th, 7th, 8th and 9th nerves with few fibers only from the 4th nerve. These appeared in the following manner: 5th nerve, between intertransversales. 6th nerve between scalenus anterior and intertransversalis passing above the rudimentary rib. 7th nerve, between scaleni anterior and medius passing immediately *beneath the tip* of the rudimentary rib. This nerve, on deeper dissection, proved to be the one which lay immediately on the rudimentary rib and was responsible for the nerve groove in figure 1. 8th nerve, between scaleni anterior and medius. 9th nerve, volume about one-half the size of the 8th nerve and three times the size of the first intercostal nerve. It joined the 8th nerve medial to the anterior border of the scalenus medius muscle and below the rudimentary rib.

The brachial plexus on the left side was formed by the 5th, 6th, 7th, 8th and 9th nerves with the larger proportion of the 4th nerve. These appeared in the following manner: 5th nerve between scalenus minimus and scalenus posterior (intertransversales deeper). 6th nerve, between scalenus minimus and intertransverse muscles. 7th nerve, between scalenus minimus and intertransverse muscles, also passing immediately above the rudimentary rib. 8th nerve, between the scaleni minimus and medius and emerged just below the tip of the rudimentary rib. 9th nerve: size about twice that of the first intercostal nerve. It joined the 8th nerve 18 mm. medial to the anterior border of the scalenus medius muscle. On neither side was there any communication of the 10th nerve with the plexus. The exact arrangement of the brachial trunks and cords had been disturbed before I was able

to examine them. There were no direct sympathetic filaments traceable to the 10th nerve.

The stellate ganglion on each side lay at the level of the neck of the first complete rib. There were numerous fine fibers running from the sympathetic chain to the periosteum of the rudimentary rib on both sides. The sinu-vertebral nerve was not found on either side.

On each side the first intercostal nerve supplied the first space by means of two twigs, the upper of which crossed the under surface of the first complete rib at about the junction of the dorsal and middle thirds of the rib. It then passed downward again to reach the anterior end of the first true intercostal space. The 20th nerve was the subcostal. The lumbar and sacro-coccygeal plexuses presented no irregularities.

#### COMMENTARY

T. WINGATE TODD

The reasons for publication of the present example of cervical rib are three in number:

First, the description aptly illustrates the particulars ascertained in the systematic investigation of the ribs of all subjects utilized in this laboratory.

Second, the specimen presents on the one side (right) a typical 'buttress' type of cervical rib and on the other (left) a 'true' rib.

Third, and the most important, is the presentation by the case of an instance where the 7th nerve, and not that formed by the combined 8th and 9th, is the trunk open to mechanical damage.

Since I wrote the previous account of certain examples of rudimentary rib (1), other articles have been published on the subject and our knowledge has considerably increased. It may be well, while discussing the present example, to include reference to other related topics which have arisen during the past two years.

In the present example there can be no doubt whatever as to what the radiographic appearance would have been, had the Department had the good fortune to possess such an apparatus.

What Dr. Gilbert Scott terms a 'true' rib would have been apparent on the left side and what he calls a 'buttress' type would have been seen on the right (4). The 'buttress' variety appearing in the skiagram as a true enlargement of the seventh cervical transverse process, however unlike the 'true' example, is nevertheless invariably a rudimentary rib, fused with the actual transverse process (5). Hence, if differentiating terms are to be used, 'fused' and 'articulating' would be more descriptive and correct than those utilized by radiographers at present.

The slope of the transverse processes of the 7th cervical vertebrae illustrates the contention of Dr. Wood Jones that it must not be relied on for a decision as to which rib is rudimentary (6).

Both rudimentary ribs in this case presented groovings for the seventh cervical nerve, a point to which reference will be made later. It would seem that as there was no connection with first complete rib or with manubrium, the lowest brachial trunks slipped down below the level of the rudimentary ribs. Indeed on the right side, as may be seen in figure 4, the seventh nerve almost succeeded in lying beneath the level of the rib, the groove produced by it occupying the ventral aspect of the bone, so that on superficial dissection the nerve actually appeared below the rib (fig. 2).

It has been stated by Wood Jones that the sulcus subclaviae never lodges the subclavian artery and that the first rib exhibits grooves for nerves and vein alone (7). With this view I am still unable to agree. The present example illustrates the grooving of a seventh cervical rib, by an artery. On the left side a channel (marked A, fig. 1) shows the site where the cervical artery crossed the bone after passing between the sixth and seventh nerve-trunks.

With regard to scoliosis, it may be pointed out that this has only been present in one of our dissected series, namely, subject C of the previous paper (1). The absence of scoliosis may possibly have had something to do with the non-appearance of symptoms in our cases; see Murphy (8).

The muscles of the present instance confirm the view that the scalenes form one mass which may be subdivided variously in



different individuals, thus giving rise to the alteration in size or or even absence of component bundles of fibers which have been dignified by special names. Here the *scalenus posterior* was large the *scalenus medius* very small: a condition the reverse of that which normally obtains. The homology of the scalenes and inter-transverse muscles is well shown; but see also (1).

The record of the subclavian arteries in this case indicates by the site and extent of the *isthmus* that the constriction was not due to muscular pressure. It is but fair, however, to observe that an *isthmus* occurred in the left deep cervical artery as it passed over the rudimentary rib, although this was not the summit of its curve (9). It is especially to be noted that the deep cervical artery was raised on the left side but not on the right. On the left side the vessel passed backward above the rudimentary rib and apparently was the artery belonging to the segment cephalad of that to which the vessel normally belongs.

It is, however, in relation to the nerve-trunks that one encounters the most striking feature of the present case.

From the clinical description of certain cases of cervical rib it is clear that in some way the seventh root has been involved and not the roots caudad of this. Or again, the symptoms indicate involvement of the seventh root in addition to the other two. This would seem to be the case in certain of the instances where symptoms first appear on the radial border of the hand. I feel convinced that in some of these the communication between the median and ulnar nerves conveys the fibers to the preaxial border. But the case described in this paper clearly shows how the seventh root may become involved in the lesion and chiefly for this reason I have urged M. Dupre to investigate the cadaver thoroughly.

The arrangement of the brachial plexus on the two sides tends to confirm Wood Jones' view that there is a definite relation between the rudimentary rib and the plexus (10) (11). Against this must be placed previous cases of mine and the fact that the symptoms may be produced by an apparently normal first rib.

## SUMMARY

1. Whatever the radiographic appearance, there is no such thing as a true enlargement of the transverse processes of the seventh cervical vertebra. The so-called enlargement is, in every instance, a rudimentary rib.

2. Certain cases which exhibit symptoms of "cervical rib" involving the seventh cervical nerve root are aptly illustrated by the present instance, in which the lowest trunk of the brachial plexus lay beneath the rudimentary rib and therefore possibly less exposed to a mechanical lesion. The seventh root on the other hand, contrary to the usual condition found in dissection, lay upon and grooved the rudimentary rib on each side.

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## A CASE OF STYLO-HYOID OSSIFICATION

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### TWO FIGURES

Stylohyoid ossification has received attention from time to time but such writings as are existent on the subject have most frequently been confined to the study of macerated specimens. There is thus still room for exact descriptions of instances which are discovered before the dissection has proceeded to any considerable extent. The present specimen was obtained in the dissecting-room of the Western Reserve University and was handed over to me for investigation by Mr. Wingate Todd. It occurred in the body of a white man fifty-five years of age, whose death was occasioned by alcoholism. As but little dissection had previously been done on the specimen, this has been a favorable opportunity, not only to describe the bony structures, but also to discuss the relation of the anomaly to the soft parts in the immediate neighborhood.

Enquiry into the clinical history of the individual did not elicit any symptoms referable to the condition, and definitely negatived any disability in speech or swallowing.

Dissection revealed a complete bilateral chain of ossicles representing the second or hyoid arch. This is shown in the accompanying radiogram. The 'prolongement hyoïdien' formed a stout irregular mass which projected some 13 mm. from the temporal bone. This was succeeded by a bar of bone, ovoid in cross-section. On the left side there was little to indicate that this was composed of two fused elements. On the right side, however, the bar displayed a thickening which reached a maximum 22 mm. from its upper extremity, and probably represented the original site of fusion.





1



2

Fig. 1 Radiogram of head of man fifty-five years of age. One-fourth natural size. The soft parts are still in position, but the occiput, vertebral column and left half of the mandible have been removed to show more clearly the position of the ossified stylo-hyoid ligaments. The pointer indicates the articulation between the tympano-hyal and stylo-hyal elements on the right side. A small backwardly directed projection from the proximal extremity of the stylo-hyal is apparent. The left stylo-hyoid ossification is not in focus; it can be seen, however, as a dark shadow passing upward and backward to the interval between the molar teeth seen on the radiogram. The body of the hyoid and the greater cornua on the right side are clearly shown.

Fig. 2 Key to radiogram. One-fourth natural size. In order more clearly to display the stylo-hyoid chain, the vertebral column, occiput and left half of the mandible were removed. The radiogram was then taken obliquely from behind. The right side of the hyoid apparatus is arranged in focus; the left side appears as a dark shadow.

On each side, the bar of bone was succeeded by a lesser horn which was fused with the hyoid bone at the junction of the body with the greater cornu, this latter having not yet become ossified in one piece with the hyoid body. The measurements of the ovoid bar were the following:

	<i>mm.</i>
Length on left side.....	70
Length on right side.....	60
Greatest diameter on left side.....	5
Greatest diameter on right side.....	6
Least diameter on left side.....	3
Least diameter on right side.....	3

The lesser cornua were mere projections of cartilage, not differentiated from that which united the body with the greater cornua.

There being some confusion over the nomenclature of the various bony elements it may be well to define exactly what is meant by the terms used. Poirier (1) adopts the following names for the four elements represented: (1) The tympano-hyal for what has above been indicated as the 'prolongement hyoïdien'; (2) The stylo-hyal; and (3) The cerato-hyal for the proximal and distal parts respectively, of the bar of bone just described. The cerato-hyal is usually represented in homo by the stylohyoid ligament. (4) The hypo-hyal corresponds to the lesser cornu.

This form of nomenclature was followed by Dwight in his recent paper on the subject (2) and is retained in this article. A different definition of terms is given by Keith (3).

Pseudo-articulations were present on both sides uniting tympano-hyal with the proximal end, and the hypo-hyal with the distal end of the long bar. The joints consisted of white fibro-cartilage with no defined joint cavity. On the other hand, the tympano-hyal on both sides was fused to the rest of the temporal bone; there was but bare indication that the long bar consisted of stylo-hyal and cerato-hyal elements; and there was no joint between the hypo-hyal cartilage and the hyoid itself on either side. Thus all the elements were ossified with the exception of the hypo-hyals. The greater cornua displayed no terminal nodules of cartilage at their free posterior extremities.

The body of the hyoid did not display the 'curved bar' referred to by Parsons as indicating the separate constitution of this bone by the 2nd and 3rd arches (4), but exhibited a median vascular foramen which may be a suggestion of the original double origin of the body. No representative of the glosso-hyal was present.

The upper extremity of the stylo-hyal on both sides presented a slight backwardly projecting process distantly reminiscent of, though probably not homologous with, the similar process on the ungulate stylo-hyal (5). The proximal ends of the stylo-hyals were 73 mm. apart. The breadth of the skull between the most prominent parts of the mastoid processes was 120 mm. The distal extremities of the cerato-hyals were 30 mm. apart; cf. Gruber's case (15).

These measurements are given to show that the processes in this case did not constrict the posterior nares as occurred in the second of Lücke's cases (6). The fused stylo-and cerato-hyals projected downward and inward. On the left side the bar of bone formed an angle of 50 degrees downward and forward and 75 degrees downward and inward. On the right side these measurements were respectively 65 degrees and 60 degrees.

The question of ossification being normal or pathological, it would be profitless to enter. The bone formation resulted doubtless from extension of the normal ossific process of the hyoid apparatus, which in such cases, as in instances of 'cervical' ribs, probably takes place at a much earlier date than used to be suspected; (for a discussion of dates see Dwight's paper). The present case is similar to that recorded by Turner but his instance was unilateral (7).

The following observations on the myology seem to be warranted:

The origin of the stylo-hyoid was, on both sides, from the posterior aspect of adjacent ends of the tympano- and stylo-hyals, by tendinous fibers.

The stylo-pharyngeus displayed an origin by muscular fibers from the same situation as did the stylo-hyoid but from the inner aspect of the process.



The stylo-glossus arose by tendinous fibers from the anterior aspect of the whole length of the stylo-hyal element. There was, naturally, no stylohyoid ligament present.

Muscles which were looked for, but of which no vestige was found, were the hyoideus lateralis and hyoideus transversus. It was thought that possibly some remnant of these muscles, so well marked in Ungulata, might be found in homo in such a case as the present.

The masto-styloideus of ungulates (8) was represented only by very dense fascia passing from the mastoid process to the tympano-hyal and adjacent extremity of the stylo-hyal on both sides of the body. It formed part of the fascial bed for the parotid gland. No fibers could be traced directly into either the stylo-hyoid or stylo-pharyngeus.

On the right side was an example of the deep stylo-hyoid of Sappey (9). It arose from midway along the posterior aspect of the stylo-hyal by tendinous fibers, gave place to a small fusiform belly, and terminated in a fibrous bundle inserted into the hypohyal. According to Macalister this muscle represents one of the muscular bands found in bony fishes (10); see also Gavarde's example quoted by Macalister (16). On the left side the deep stylo-hyoid muscle was not found.

The relation of the styloid process to the bloodvessels confirmed the description already given by Dwight. On palpation through the mouth, the elongated stylohyoid process could be felt on each side, immediately below and behind the tonsil. In the cadaver it produced a distinct, though but slightly elevated ridge where the base of the tongue formed the anterior boundary of the vallecula. Dissection showed it to lie on the outer aspect of the bucco-pharyngeal fascia and distant by 5 mm. from the oral pharyngeal mucous membrane. Its disposition was therefore similar to that of the process recorded by Kyle in his case of fractured styloid (11).

There was no constriction of the posterior nares. It would seem from Kyle's case, as from the present one, that the ossified ligament may produce no symptoms. In Kyle's case temporary

aphonia, a sensation of constriction in the throat when singing or speaking and loss of the singing voice resulted from fracture of the process.

How far complete ossification of the styloid process may be responsible, in certain cases, for clinical symptoms, is not yet clear. Richardson records an instance of bilateral long styloid with which occurred post-nasal catarrh, soreness in the tonsillar region, difficulty in swallowing and soreness of the neck after much use of the voice. Moreover, the symptoms were cured by removal of the process (12).

Bigelow's case of the 'clicking woman' mentioned by Dwight, may have been an instance of ossified stylo-hyoid ligament (13). I have not thought it necessary to dwell upon those instances and clinical complications already referred to in detail by Dwight (2).

The position of the process in relation to the tonsil may be variable according to the length and direction of the bone. In Richardson's case already mentioned, a shorter process than the one recorded here, lay just within the anterior tonsillar fold and parallel to its course.

It is well to be on one's guard against the possible presence of the subpharyngeal cartilage of Luschka when making a clinical diagnosis of elongated styloid process. This is emphasized by Wingrave who mentions that besides occurring in the lateral wall of the pharynx, somewhat below and behind the faucial tonsil, the cartilage may also be present in the tonsil itself and in the latter situation, will give resistance to the guillotine (14).

In conclusion, I would express my obligation to Prof. T. Wingate Todd for his help throughout the investigation and in the writing of this article.

SUMMARY

1. Complete ossification of the stylohyoid ligament may occur without the production of any clinical symptoms.

2. Associated with the anomaly, muscular variations may be present.

3. A frequent site of such an ossification is below and behind the tonsil in the anterior part of the vallecula where it may be palpated clinically.

4. The situation may vary, especially when the ligament is not ossified throughout its length. In such a case it may lie in front of the tonsil.

5. Constriction of the posterior nares or oral pharynx does not occur unless the process is markedly deflected.

6. In making the clinical diagnosis of ossified stylohyoid ligament, the occasional presence of the sub-pharyngeal cartilage of Luschka must be remembered.

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## A CASE OF MULTIPLE RENAL ARTERIES

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ONE FIGURE

While cases of multiple renal arteries are numerous, occurring in 43 per cent (Macalister '83) of bodies examined, explanations for their occurrence have depended on insufficient embryological evidence, and have been therefore, largely speculative. Although the present case adds no new fact to our knowledge of the condition, its publication in the light of recent studies (Evans '09, (Jeidell '11) is of embryological interest.

During the regular course of instruction in dissecting, my attention was drawn to the abnormal appearance of the kidneys of a white male subject, No. 293. The clinical diagnosis was chronic interstitial nephritis. Both kidneys lay at their normal levels, and in their relative positions. The extremitas inferior was drawn closer to the mid-line than the superior, apparently by several super-numerary blood vessels. In shape the kidneys were much altered from the normal, the right being oval and flattened, the left pear-shaped with the larger end cephalad. On both organs the hilus was placed on the ventral surface. The anterior lip was represented by a low ridge curving laterad across the ventral surface of the kidney and approaching the caudal pole. The normal sinus renalis was absent, the vessels, nerves, and calyces penetrating a broad, smooth, convex surface continuous with the surface of the kidneys caudad and mesad. The posterior lip of the hilus was absent.

The abnormal form and position of the kidneys in association with accessory renal arteries has been pointed out by numerous observers. In many cases the kidneys preserve their foetal lobulation (Poirier, Broedel, '01). In others the hilus remains on the

ventral surface of the gland instead of rotating to the inner border as normally (Tyrie, Young and Thompson). In general the kidney deviates from the normal reniform shape in proportion to the number of the vessels. Tyrie believed that a moulding of the kidney by the abdominal parietes together with rotation of the gland by increased arterial pressure modifies its shape, while Young and Thompson thought changes in shape are due to the differences in nutrition varying according to the irregular arterial supply. On the other hand, Rupert ('13) in thirty-five out of fifty cadavers, found anomalies of the renal arteries without any change in the normal positions of the kidneys, although their shapes were altered. The present case well illustrates the departure from the usual reniform shape, the displacement of the hilus in both glands from the mesial border to the ventral surface, and the undeveloped character of the labia of the hilus. Besides the influence on the rotation of the glands which increased arterial pressure or differences in nutrition may exert, there must be considered the possible mechanical effect of persisting anastomosing vessels derived from the primitive plexus supplying the kidneys. The factors which influence the selection of certain channels of the capillary plexus and the elimination of others remains for future research.

An examination of the kidneys and their vessels removed from the abdominal cavity with portions of the aorta and vena cava showed a normal *A. renalis sin.* arising from the lateral aspect of the aorta immediately below the *A. mesenterica superior* and entering the hilus at its cephalic extremity on the ventral surface of the kidney, giving off three branches, one of which, the longest, descends to the middle of the gland before entering, lying dorsad to the normal *V. renalis sin.* and the pelvis; the smaller branches lie in front of these structures as shown in figure 1.

The normal renal artery is formed from the lateral row of primitive aortic branches which supply the mesonephros, terminating in a plexus lying between the reproductive gland, the mesonephros, and the metanephros (Felix '12). Hochstetter ('91), Pohlman ('05) and Hill ('05) stated that the kidney does not receive its renal artery until it reaches its definitive position, but



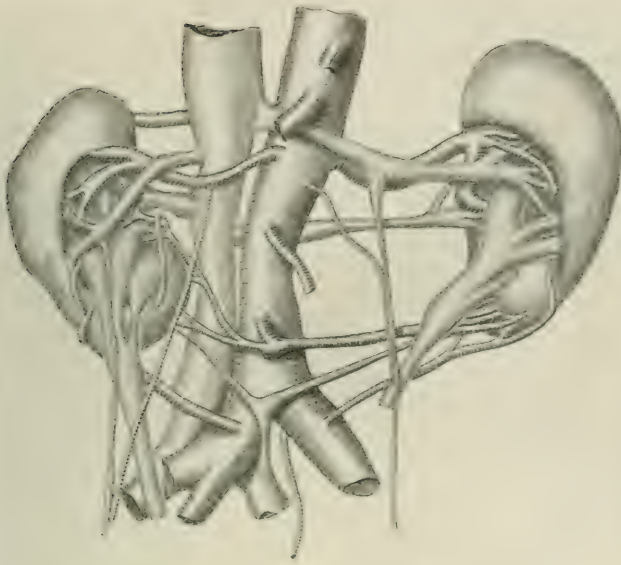


Figure 1

by the use of the embryos more completely injected in the younger stages than those used by previous observers, Evans showed an early metanephric plexus arising from the A. sacralis media, later confirmed by Jeidell who showed also a source for the plexus from the A. mesenterica inf. The vascular supply to any organ, as shown by Evans, spreads by capillaries and not as outgrowths of the permanent vessels from the main vascular trunks. The kidney vascular supply accordingly is derived by retention of certain capillary vessels of the embryonic renal plexus. Broman (1906) derived the normal renal arteries from the mesonephric arteries, and Felix observed that as the kidney migrates upward and the cephalic vascular supply by way of the mesonephric arteries becomes sufficient, the caudal branches separate from it, until, when the kidney has reached its definitive position, only one persists as the permanent renal artery. If, then, one or more of these caudal branches persist they may become accessory. Others have explained multiple renal arteries as branches of the renal artery

arising sooner than normal, the origin of the branches having travelled in, as it were, and come to arise separately from the aorta instead of from one common stem. Kolster ('01) stated that multiple renal arteries are derived through premature division of the single normal renal artery, or they may be persisting branches of the renal artery which normally degenerates in embryonic development, but which through some unknown, accidental disturbance during development remain accessory. The latter explanation is similar to Macallister's who considered multiple renal arteries as persisting enlargements of branches which normally exist as fine extra-peritoneal anastomoses between the capsular arteries and those supplying the abdominal wall. Young and Thompson ('03) suggested that multiple renal arteries are produced by arrested development of the kidney. These speculations have now given way to the embryological explanation based on the evidence of injected embryos. Multiple renal arteries are persisting embryonic vessels of the capillary plexus supplying the normal embryonic kidney. The anomalies which follow are readily explained on this basis.

On the right side the largest renal branch of the aorta slightly higher than the A. renalis sin. passes behind the V. cava inf. and enters the cephalic extremity of the right kidney at the median border. It does not branch. This apparently is a persisting mesonephric artery of the group which lies dorsad to the embryonic supra-renal body, and therefore enters the kidney more dorsad and nearer the cephalic extremity, than the other vessels (Felix). Just below this branch and arising from the ventro-lateral aspect of the aorta is a large branch which enters the hilus of the right kidney at its cephalic extremity after crossing the ventral surface. It breaks up into three branches which lie in front of the pelvis and vein. It gives off the A. spermatica interna. This branch is possibly a persisting mesonephric artery of the group which passes through the primitive suprarenal body (Felix). The A. spermatica interna arising from an A. renalis dex. is not unusual, being derived from a mesonephric vessel, which as in this case, anastomoses with branches entering the hilus, and finally becomes of less importance than the anastomosing vessels.

Midway between the *A. mesenterica inf.* which is normal and the bifurcation of the aorta there arises from the ventral surface of the aorta a trunk about 1 c.m. in length, giving rise to right and left accessory renal arteries. The branch to the right kidney almost immediately bifurcates, one branch coursing cephalad along the median border of the gland to enter the ventral surface dorsad to the pelvis, the other branch entering the caudal pole directly. The branch to the left kidney passes dorsad to the ureter and pelvis and enters the caudal pole of the gland by numerous radicals. Portal described a case in which both renal arteries arise from a common stem originating from the front of the aorta. The anomaly is probably due to the apposition of opposite ventral branches of the primitive aortae coincident with the fusion of these vessels, resulting in *rami intestinales* which have anastomosed with the early renal capillary plexus.

From the *A. iliaca communis dex.* there arises from the ventral aspect a large branch crossing ventrad to the bifurcation of the *V. cava inf.* and dorsad to the ureter and spermatic vessels, and gaining the dorsal surface of the right kidney to which it supplies a large branch. Then winding across the lateral border and over the anterior lip of the hilus it enters the ventral surface dorsad to the pelvis. This anomaly has been described frequently. It is the retained embryonic blood supply of the gland while in its pelvic position, probably through *rami intestinales* anastomosing with branches of the capillary renal plexus.

From the ventral aspect of the *A. iliaca communis sin.* there arises a branch smaller than the one just described, pursuing a course corresponding to that of the latter artery, and distributing itself in a similar manner. Its origin may be explained in the same way.

A normal *V. renalis sin.* arises by four radicals from the ventral surface of the left kidney, crosses the aorta to the *A. mesenterica inf.* receiving the *V. spermatica int. sin.* and enters the lateral aspect of the *V. cava inf.* Caudad to this vein and nearer the median border arises a large vein from several radicals, which passes dorsad to the aorta, and enters the dorsad lateral aspect of the *V. cava inf.*, receiving in its course a large vein, *V. azygos*



minor. This anomaly is similar to the examples reported by Froriep ('95), who considers it due to a cross anastomosis between the left and the right cardinals, the latter having become the post renal portion of the V. cava inf.

The largest vein from the right kidney arises by numerous radicals from the ventral surface of the glands, receives a branch of small calibre from the caudal pole, and enters the lateral aspect of the V. cava inferior caudad to the level of normal V. renalis sin.

The V. renalis dex. arises from the ventral surface near the anterior lip of the hilum by several radicals, receives the V. spermatica int. dex., crosses ventrad to the A. renalis dex., then cephalad to it, and enters the ventro-lateral aspect of the V. cava inf.

The caudal poles of both kidneys are drained by small veins joining at the level of the bifurcation of the aorta to form a common trunk 2 cm. long entering the ventral aspect of the V. iliaca transversus. This anomaly is especially interesting because of its rarity.

The accessory renal veins, like the multiple arteries may be explained as persisting branches of the embryonic renal plexus of veins. Undoubtedly, insufficient attention has been devoted by anatomists to multiple renal veins which are as important to consider as arteries, especially in nephrectomy, (Rupert). The present case shows them to be quite as unusually placed in their relations as are the arteries.

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## COMMUNICATIONS FROM SCIENTIFIC INSTITUTIONS

### *Biologische Versuchsanstalt der Kaiserlichen Akademie der Wissenschaften in Wien.*

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<sup>1</sup> A separate department of plant physiology with W. Figdor as director is provided for.



## THE MORPHOLOGY OF THE LONG ACCESSORIUS MUSCLE

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### FOUR FIGURES

In the dissecting-room of this School during the past winter session we have obtained four examples of the long accessorius muscle. We have thought it advisable to place these on record as they form a useful summary of the various origins of this muscle, when present, and of its relation to the accessorius muscle of the sole (*M. quadratus plantae*).

Many previous observations on this muscle are to be found, in brief, in the accounts by Le Double (1) and Testut (2). We have, however, endeavored to review the subject afresh, in order to present in concise form the views at present held regarding the origin of this muscle. Our personal investigations have been carried out on twenty-five cadavera, among which only two revealed the presence of the muscle. We have also dissected the myology of the hindlimb in the following animals: *Macacus rhesus*, *Tatusia peba*, *Alligator mississippiensis*.

In this way we hoped to obtain information of assistance in interpreting the muscular arrangement in homo. We do not propose at present to publish the result of these dissections, but simply to extract parts which have direct bearing on the subject under discussion. We would acknowledge our indebtedness to Dr. T. Wingate Todd for placing the material at our disposal and for assistance throughout the investigation. The specimens of *Macacus rhesus* were generously presented to the Department by the authorities of the Cleveland Zoological Gardens.

## DESCRIPTION

*Instances I and II from Subject 125, mulatto, male, aged forty years*

*Instance I: Right side (fig. 1).* The muscle arose by two heads. The long head possessed a tendinous origin, 3 cm. long, partly from the medial subcutaneous border of the posterior aspect of the tibia and partly from the adjacent fascia covering the M. flexor digitorum longus, 20 cm. above the medial malleolus. The tendon gave place to a flat muscular belly 10 cm. long and 1 cm. broad. The belly passed into a narrow tendon which entered a compartment on the postero-internal aspect of the ankle, medial to that for the M. flexor hallucis longus. The short head possessed an aponeurotic origin 3 cm. broad from the lower and lateral part of the fascia of the leg deep to the tendo calcaneus and also from the medial aspect of the calcaneus in this way being continuous with the medial head of origin of the M. quadratus plantae. The short head formed a flat muscular sheet inserted into the tendon of the long head.

The common tendon, thus constituted, divided to be inserted into the tendons passing to the 3rd and 4th digits from the M. flexor digitorum longus.

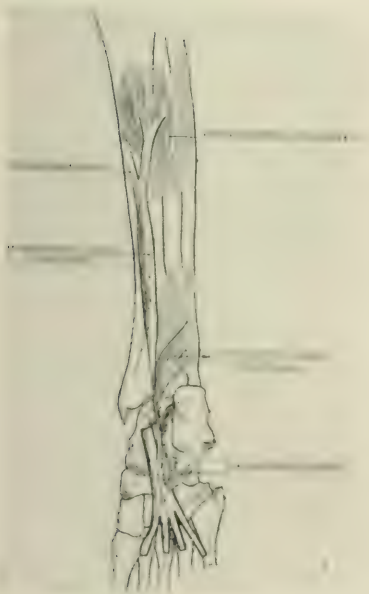
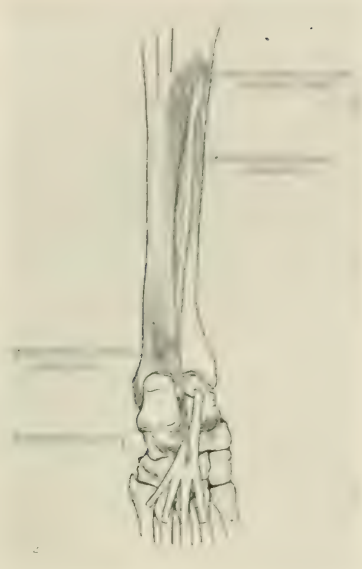
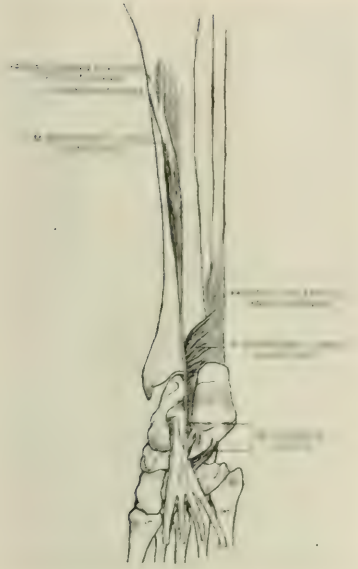
*Instance II: Left side (fig. 2).* Long and short heads of origin were present. Except that their origins were from fascia only, both long and short heads corresponded in all particulars with those on the right side. The origin of the M. quadratus plantae extended higher than normal on the medial aspect of the calcaneus but a hiatus of 5 mm. breadth separated it from the short head of the accessorius longus.

Fig. 1 M. accessorius longus arising from tibia, fascia covering M. flexor digitorum longus and from fascia of leg deep to tendo calcaneus. The muscle is continuous with the M. quadratus plantae. One-fifth natural size.

Fig. 2 M. accessorius longus arising from fascia only (cf. origins in fig. 1). Short head not continuous with M. quadratus plantae. One-fifth natural size.

Fig. 3 M. accessorius longus arising from tibia, fascia superficial to M. soleus, and from fascia of leg deep to tendo calcaneus. Short head not continuous with M. quadratus plantae. One-fifth natural size.

Fig. 4 M. accessorius longus arising from fascia covering M. flexor digitorum longus and from fibula. Short head of muscle not continuous with M. quadratus plantae. One-fifth natural size.





*Instances III and IV from Subject 89, negro, male, aged twenty years*

*Instance III: Right side (fig. 3).* The muscle arose by two heads. The long head was constituted by two fibrous slips, the one from the medial margin of the tibia, as in Instance I, the other from the fascia superficial to the M. soleus about 18 cm. above the medial malleolus. Thus about 2 cm. of the belly of the M. soleus lay between the two slips of origin. The belly of the long head presented a flattened appearance: its length was 12 cm. and its breadth 1 cm. The fibers converged to a slender tendon which passed behind the ankle to the foot, bearing the same relation to the tendon of the M. flexor hallucis longus as in Instance I and receiving in its course some muscular fibers which constituted the short head. These fibers had an aponeurotic origin from the fascia of the lower and lateral part of the leg deep to the tendo calcaneus and about 2 cm. above the medial malleolus.

The short head was not continuous with the origin of the medial head of the M. quadratus plantae. The fibers of the last-mentioned muscle joined the tendon of the M. accessorius longus, and distal to this the composite tendon was inserted into the superficial aspect of the undivided tendinous mass of the M. flexor digitorum longus.

*Instance IV: Left side (fig. 4).* Again the muscle presented two heads of origin. The long head arose by aponeurotic fibers, 1.5 cm. in length, from the fascia superficial to the M. flexor digitorum longus, 13 cm. above the medial malleolus. The belly assumed a cylindrical appearance, was 8 cm. long and 1 cm. broad, and terminated in a long slender tendon.

The short head formed a flat fan-shaped muscular sheet 5 cm. broad at its origin, by muscular fibers directly from the posterior aspect of the fibula, the lower border of the origin being 2 cm. above the groove for the tendons of the MM. peronei. The fibers of the short head converged to an insertion 3 cm. broad along the slender tendon of the long head. The common tendon, thus formed, was 8 cm. long and partially fused with the common tendinous mass of the M. flexor digitorum longus, from which it separated again and finally fused with the flexor longus tendons

passing to the 2d, 3d and 4th toes. There was a considerable interval between the short head and the medial origin of the *M. quadratus plantae*.

In both subjects, the *MM. plantaris*, *flexor digitorum longus*, and *flexor hallucis longus* presented no variations worthy of note.

#### *Nerve-supply*

Only in Instance III was it possible to identify the nerve-supply of the muscle. In this case the nerve to the long head was given off from the *N. tibialis* in the upper third of the leg. Unfortunately, at the period of dissection, when the muscles were discovered it was not possible to identify the nerve-supply of the *M. quadratus plantae*. We had thought that these cases might have exhibited a nerve-supply from the medial plantar nerve, as described by Poirier (3), rather than that from the lateral plantar nerve as usually indicated in textbooks.

#### DISCUSSION

The cases cited above confirm the established teaching that the long accessorius is a vestige of an ancestral muscle of some size, the lowest portion of which alone remains to us as the *M. quadratus plantae*, and which was, in its development, entirely independent of the *M. plantaris*.

Typical instances of this independence of the *MM. accessorius longus* and *plantaris* are given in Morrison Watson's description of the muscular anatomy of *Proteles* and *Hyaena* (4).

There is evidence to show that the *accessorius longus* muscle is developed in connection with the *M. hallucis longus*. An instance of this is given by Parsons in a specimen of *Gymnura rafflesii*, in which the *flexor accessorius* formed a muscular bundle arising from the lateral surface of the calcaneus, crossed the *flexor* tendons to the toes, from which it received a few fibers and was inserted into the terminal phalanx of the hallux, in this way replacing the *flexor hallucis longus* (*fibularis*) (5).

A less extreme form is illustrated by Young's specimen of *Viverra civetta* (6). Such an idea is fostered by the observation

of Schomburg in an early human embryo of the fusion of the MM. quadratus plantae and flexor hallucis longus (7). But against this view must be set the fact that Bardeen did not confirm Schomburg's observation (8), and also that McMurrich believes the M. accessorius to be associated more particularly with the tibial than with the fibular flexor (9). Gegenbaur in former years considered the M. quadratus plantae to be a derivative from the muscular mass giving origin to both tibial and fibular flexors (10). Eisler thought the muscle rather to be associated with the tibialis posterior and to have no direct connection with either flexor (11).

Erna Glaesmer, after her investigation, could come to no conclusion but did not agree with Gegenbaur's interpretation (12). Later she states that she found the M. quadratus plantae arising in some marsupials from the fibula and not from the calcaneus (13).

The derivation of the muscle cannot therefore be said yet to be settled beyond dispute. But its connection with the flexors of the digits in the leg and with the tibia and fibula find parallels among lower animals.

So likewise may its connection with the fascia covering the soleus be harmonized with the similar occurrences in Mammalia. For while Parsons found no connection between the 'accessorius' and soleus in *Choloepus* or *Bradypus* (14), yet Humphry described the occurrence in certain instances of a blending between these muscles (ref. 15, p. 177). Here it may be mentioned incidentally that in our specimen of *Tatusia peba*, we were able to confirm Bland Sutton's description of the M. plantaris (16) but failed to find the condition described by Macalister (17).

Our specimens do not justify us, at present, in entering into a discussion of the variations of insertion of the M. quadratus plantae. This subject has been recently referred to by Miss Glaesmer (13).

With regard to the morphological variations in the tendons of insertion of the tibial and fibular flexors we can add nothing to the accounts already given by Humphry (15), Keith (18), Glaesmer (13) and others.



## SUMMARY

1. Although there is considerable divergence of views regarding the origin of the long accessorius, there is some evidence to show a close association with the *M. flexor digitorum longus*.

2. The various origins of the crural portion of the *M. quadratus plantae* find their counterparts in lower animals.

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## COMMUNICATIONS FROM SCIENTIFIC INSTITUTIONS

### *Internationaler Kongress für Vererbungs- und Züchtungsforschung.*

Einem Beschluss des auf dem letzten Kongress in Paris gewählten internationalen Ausschusses zufolge wird der nächste Kongress im Jahre 1916 in Berlin abgehalten werden. Die Einladung nach Berlin ist ergangen von einem in Berlin zusammengetretenen "Engeren Ausschuss zur Vorbereitung des 5. internationalen Kongresses für Vererbungs- und Züchtungsforschung." Dem Ausschusse gehören an Wirkl. Geheimer Rat Dr. Thiel Exzellenz, Präsident der Deutschen Gartenbaugesellschaft, als Vorsitzender, sowie die Herren Geheimer Ober-Regierungsrat Dr. Boenisch und Gerichtsassessor Dr. Kniebe als Vertreter des Herrn Staatssekretärs des Innern, Geheimer Oberregierungsrat Ministerialdirektor Dr. Schröter und Geheimer Regierungsrat Dr. Oldenburg als Vertreter des Herrn Landwirtschaftsministers, Prof. Dr. Krüss als Vertreter des Herrn Kultusministers, Kammerherr v. Freier-Hoppenrade (Vorsitzender der Deutschen Landwirtschaftsgesellschaft), Ökonomierat Hösch (Vorsitzender der Deutschen Gesellschaft für Züchtungskunde), L. Kühle (Vorstand der Gesellschaft zur Förderung Deutscher Pflanzenzucht), Geh. Regierungsrat Prof. Dr. v. Rümker und Prof. Baur. Der Kongress soll Anfang September 1916 stattfinden. Geschäftsführer des Vorbereitungsausschusses sind die Herren Baur und v. Rümker.

Die Adresse des Ausschusses ist: Berlin N 4, Invalidenstrasse 42, Kgl. Landwirtsch. Hochschule.

## A CASE OF ATRESIA ANI IN A HUMAN EMBRYO OF 26 MM.

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### ONE FIGURE

Among the most common anomalies of the rectum are those of atresia ani, atresia recti, and atresia ani et recti. The first of these is described as being present when an anal opening is lacking. An anal invagination may or may not be present, or the site of the anus may be marked by a surface elevation. In atresia recti simplex the anal opening is present and normally developed, but the upper part of the rectum, failing to unite with the anal portion, ends blindly in the pelvis. In atresia ani et recti, both anal opening and a portion of the ampulla recti are missing. These simple atresiae may be complicated by the additional anomalous conditions of fistulae into the urethra, bladder, vagina, vulva, or through the scrotum and perineum.

Such anomalies of the rectum, which are usually detected at the time of birth or shortly afterward, have aroused considerable interest, and various theories have arisen as to their probable origin. Among those who have studied the causes of rectal anomalies should be mentioned the names of Rotter ('03) and Jones ('04).

However correct any of the theories regarding the formation of these atresiae may be, before they can be definitely accepted, they demand not only a correct knowledge of the normal development of these parts, but direct evidence concerning the time and manner of deviation from the normal. It is because of this that the present atresia, found at a comparatively early stage of the embryo, is described. Although the author knows of no other case of this kind found in an embryo, the observation which Keibel



made on an embryo of 11.5 mm. should be mentioned. He found that the lumen of the rectum in its lower part had become occluded at two small places. The observation, however, offers no evidence, direct or indirect, that this would develop into a case of atresia. It seems probable that the lumen might have again opened up, for in corresponding stages of pig and rabbit embryos (Lewis '03) and of chick embryos (Minot '00), where there is normally an epithelial occlusion of the rectum, such reopening occurs. It should also be recalled that epithelial occlusion occurs normally in the developing duodenum (Tandler '00, Johnson '10).

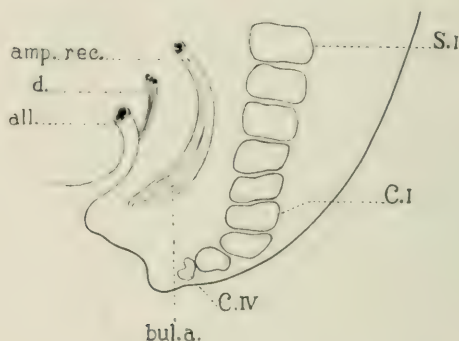


Fig. 1 Graphic reconstruction of the pelvis of a human embryo of 26 mm. (H 99). *all.*, allantois; *amp.rec.*, ampulla recti; *bul.a.*, blindly ending bulbus analis; *d.*, Müllerian and Wolffian ducts; *C.I.* and *C.IV*, first and fourth coccygeal vertebrae; *S.I*, first sacral vertebra.

The case of atresia under consideration (see accompanying figure) was found in Embryo H 99 of Prof. C. M. Jackson's collection, the crown-rump length of which is 26 mm. The sections were cut transversely to the long axis of the embryo, hence the plane of sectioning through the lower part of the rectum is longitudinal. The upper part of the rectum is apparently normal. Its epithelial tube has a transverse diameter of 0.23 mm. and is composed of two to three layers of cells. More caudally the rectal tube expands, forming what has been termed in a former paper (Johnson '14) the bulbus analis. This has a transverse diameter of 0.29 mm. at its widest place and its epithelium is of about

the same thickness. A single deep infolding extends itself along the ventral wall of the tube. The epithelium of the upper part of the bulbus analis is regular, its nuclei are closely packed and distinct, but cell boundaries are not distinguishable. At about the middle of the bulbus analis the epithelium becomes broken up, and from this point caudally becomes more and more irregular. In this region the nuclei are more scattered and in places less sharply outlined than above. The lumen becomes gradually filled up with broken-off pieces of the epithelium, and lower down becomes lost in a mass of epithelial debris. The lower portion of the bulbus analis is smaller in size, and finally disappears from view altogether. The last remnants are seen as a few nuclei scattered about in the mesenchyma. The epidermis of the anal region is found about 0.55 mm. caudad to these last remnants. An anal invagination is present but it is very shallow.

Surrounding the epithelial tube throughout its whole length is mesenchyma. In the upper part of the bulbus analis this lies close to the epithelium, but in the lower part, where the epithelium is broken down, the mesenchyma is separated from it by a distinct shrinkage space. At the termination of the epithelium the shrinkage space again disappears. Between the lower end of the rectum and the anal invagination, the mesenchyma shows no special features. It is possible that some of the nuclei seen in the mesenchyma of this region belong to the epithelium, but this point is not determinable.

The inner circular and the outer longitudinal layers of the muscularis are distinct. They terminate a short distance above the last remnants of the epithelium. As has been formerly shown (Johnson '14) the circular muscle coat terminates normally at the constriction between the bulbus analis and the bulbus terminalis. This affords a means of determining that the last portions of the epithelium represent a part of the bulbus terminalis.

In front or ventral to the rectum is seen the urogenital sinus, which at this stage is quite widely separated from the rectal tube. Opening into it are seen the united Mullerian ducts and on either side, the two Wolffian ducts, although their epithelia are somewhat broken up and irregular. The ureters do not join the uro-

genital sinus but end blindly, their epithelium being broken down in a similar manner to that of the rectum.

The results of my work on the development of the normal rectum have shown that the bulbus analis and the bulbus terminalis go into the formation of the zona columnaris and zona intermedia of the pars analis recti respectively. Since in this anomalous embryo the epithelium of the rectum terminates in the region of the bulbus terminalis, it is evident that the missing portion of the rectal tube represents what would have normally been the zona intermedia. The anomaly should, therefore, be classified as a case of atresia ani simplex. However, owing to the peculiar disposition of the ureters, it is doubtful whether it would have retained its simple relations, had the embryo lived longer.

Regarding the cause of this anomaly little can be definitely said. It is doubtful whether the anal membrane is in any way concerned. In an examination of 19 embryos of 15.5 mm. and over, Keibel and Elze ('08) show that in stages of 15.5, 16, 17, 18, 18, 18.5, 19.5, 20, 20, 20, 20, 20.5, 22, 22.5, 24, 24, 26 and 26 mm. the anal membrane was present and the anal passage closed to the outside, while in only one case (22.5 mm.) was the passage open. Broman ('11) states that an anal opening is not effected until the embryo is about 33 mm. in length. The results of my own work show that the anal membrane is present in embryos of 17, 19 and 22.8 mm. and has disappeared in those of 16, 29, 30, 30 and 31 mm. and above. From these observations it will be seen that usually at 26 mm. the anal membrane occludes the anal passage, so that the above anomaly cannot be explained by a failure of the anal membrane to break down normally. Constriction and a subsequent breaking apart of the epithelial cord at this place must have occurred.



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The Index Office which has recently been established in Chicago intends to make a specialty of serving the medical profession by undertaking to supply exhaustive or selected bibliographies of medical subjects, translations or abstracts of articles or monographs, copies, photographic or otherwise, of manuscript, printed or illustrative material.

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Dr. Bayard Holmes, surgeon and medical writer, is President of the Office, Aksel G. S. Josephson, Cataloguer of the John Crerar Library, is Secretary and directing officer. The Office is located at 31 West Lake Street, Chicago.

## CYCLOPIA IN MAMMALS

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TWELVE FIGURES

Among the mammals, cyclopia has been reported in man, monkey, cow, sheep, goat, pig, stag, horse, dog, cat, rabbit. In this paper is recorded a monster of the cyclopean order in the rat. Cyclopia has also been recorded in birds—in the chick, goose, dove, duck; and in fish—in the salmon, skate, trout. Cyclopean monsters have been produced experimentally in the chick, frog, salamander, fish and flatworm.

In many of the cyclopean monsters there are no abnormalities other than those of the head. In some, the mouth is normal. The oldest cyclops in man, recorded by Valenti ('94), lived 73 hours. In the skate described by Paolucci ('74), the age was several years, since the fish was  $1\frac{1}{2}$  feet long and  $2\frac{1}{2}$  feet wide. The fish seemed to be able to procure its food readily. Yung ('01) reports a case of monophthalmia in a rainbow trout. The eye was on the left and apparently normal. The fish lived 22 months, feeding on Tubifex; movement was in circles. Stockard ('10) succeeded in keeping magnesium embryos for 1 month in aquaria. Many of the human cyclops breathe in a shallow way through the mouth, for only a few minutes.

This paper is a study of three specimens of the mammalian cyclops: (1) Cyclopean rat: Two monsters appeared in the mother's first litter of six rats. One specimen was a case of anterior hydrencephalocoele and will be described elsewhere. The other was a case of anophthalmia cyclopica. For these two specimens and two normal members of the same litter, I am indebted to Dr. Newton Miller. (2) Cyclopean pig with otocephalus: This rare specimen was loaned to me by Prof. F. R. Lillie of the University of Chicago. The specimen came from the Chicago stockyards. (3) Human cyclopean brain: This specimen was sent me by Prof. H. H. Wilder of Smith College, who has already described the external anatomy and the structure of the eyeball. The specimen was No. 6956 of The Wistar Institute Collection. I am indebted to Dr. Greenman of The Wistar Institute and to Professor Wilder for the privilege of studying the brain and for information regarding the fetus.



*External appearance of the specimens*

The rat was slightly smaller than the two controls, measuring only 30 mm. while they were each about 40 mm. long. The specimen was a female and except for the absence of eyelids and eyeballs, seemed to be normal.

The cyclopean otocephalic pig (fig. 1) was a female and measured 165 mm. C. R. The bitemporal diameter of the head was 45 mm. There was no proboscis and no eyeball. In the middle of the forehead there were three eyelids; the upper one was composed of the two fused



Fig. 1 Cyclopean otocephalic pig.

upper lids; under the eyelids, which together were 5 mm. in diameter, was a pit 1 mm. in diameter, which might possibly be interpreted as the beginning of the invagination of the ectoderm to form a lens.

The human cyclops, from which I obtained the brain only, was a male, with nasal proboscis about  $1\frac{1}{2}$  inches long, and a double eye of the hour-glass type. Professor Wilder has described the specimen (Wilder '08) as a "typical human cyclops, identical in general appearance with the specimen photographed by Hirst and Piersol, vol. 3, plate 31."

## COMPARATIVE STUDY OF THE SPECIMENS

*The rat*

The head of the rat was dissected under a Zeiss binocular and the brain was exposed and studied *in situ*, then removed, drawn in several positions, and finally embedded in paraffin and sectioned. The sections were from 15 to 20 micra thick and the stains used were congo red and hematoxylin-eosin.

*Skull.* The skull bones were a little thicker than in the controls, but were not seriously displaced. The frontals were fused and no trace of the nasals was found.

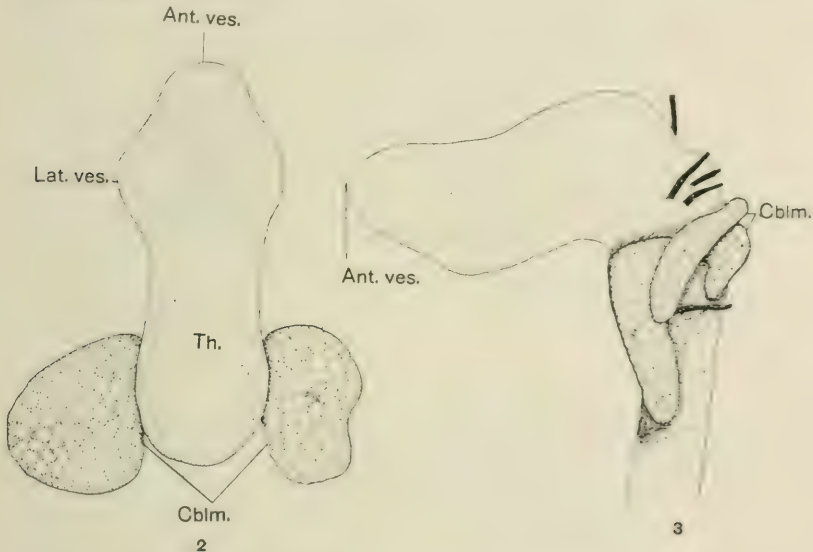


Fig. 2 Dorsal view of the brain of an anophthalmic rat. *Ant. ves.*, anterior lobe of cerebral vesicle; *Cblm.*, cerebellum; *Lat. ves.*, lateral lobe of cerebral vesicle; *Th.*, thalamus.

Fig. 3 Lateral view of the brain of the anophthalmic rat.

*Brain and cerebral nerves (figs. 2 and 3).* A study of the brain in gross showed that there were three cerebral vesicles, one being slightly anterior to the two paired lateral vesicles. There was no external indication of hydrocephalus. The cerebellum was pushed back to a ventral position and the hemispheres were somewhat separated from the vermis. The cerebral peduncles were absent; the cranial nerves anterior to the fourth were absent; the thalamus was well developed and the fourth ventricle was normal; the corpora quadrigemina were apparently normal. On comparison with the normal brain, it was found that the brain of the monster measured 5 mm. in length from the anterior end

of the cerebral vesicle to the cervical flexure, while the control brain measured 9 mm. in a corresponding region; the control brain measured 7 mm. through the cerebral hemispheres, while the brain of the anophthalmic rat measured but 2 mm. through the lateral vesicles.

The study of serial sections showed the apparent absence of the corpus callosum. The ventricles, however, showed only a slight enlargement, without a marked hydrocephalus; the hypophysis was present, but the epiphysis was absent, or if present it was not discovered.

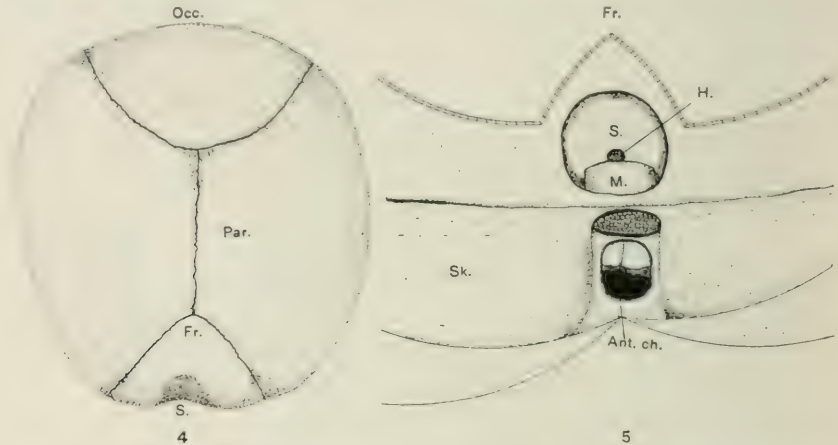


Fig. 4 Skull of the cyclopean otocephalic pig. *Fr.*, fused frontals; *Occ.*, occipital; *Par.*, parietal; *S.*, eye-socket.

Fig. 5 Front view of the skull of the pig, with the skin rolled down to the mouth and the eye-muscles cut transversely. *Ant. ch.*, anterior chamber of the eye; *Fr.*, cut frontals; *H.*, glandular sac; *M.*, cut eye-muscles; *S.*, eye-socket; *Sk.*, skin rolled down to expose eye-socket.

### *The pig*

In this extremely rare monstrosity, the ears and mouth were fused, with a single opening but 5 mm. in diameter; no proboscis was present. It is interesting to note that the only case of a human fetus with the proboscis below the eye, described by Allan ('48), was a case of cyclopean otocephalus.

*Skull (fig. 4).* Since it was desired that the specimen be as little mutilated as possible, not all the bones of the skull were examined. The frontals were fused, the parietals and the occipital were in about normal position, but the parietals extended anteriorly for some distance and joined the frontals in the region ordinarily occupied by the nasals. The lower jaw was absent.

*Eye-socket and eye-muscles (fig. 5).* After removing the skull-cap, an attempt was made to expose the face down to the mouth, without



injuring the specimen for museum purposes. The skin was carefully rolled down to the mouth, a transverse cut being made through the mass of eye-muscles; figure 5 shows the region exposed. There was no indication of a lens or eyeball; a glandular sac was present just above the attachment of the eye-muscles to the base of the eye-socket. This might possibly be interpreted as a portion of the hypophysis. The little pit under the eyelids (fig. 1) was examined from the rear but no

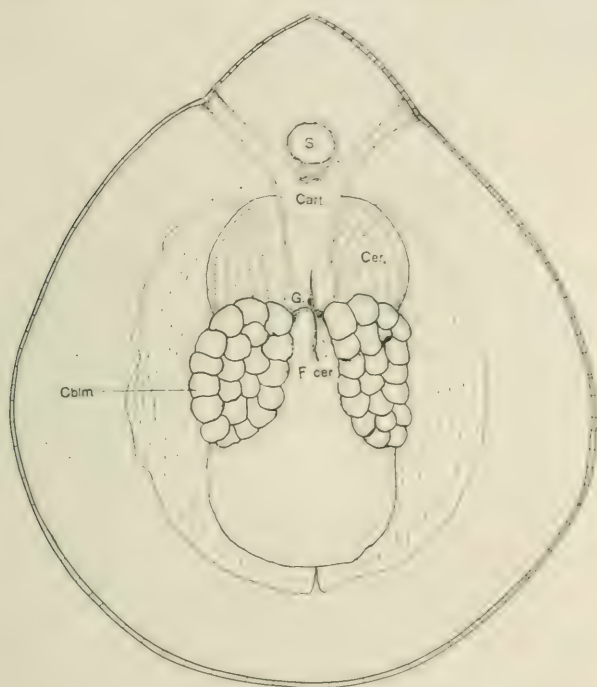


Fig. 6 Brain of the cyclopean pig in situ. *Cart.*, displaced nasal cartilage; *Cer.*, thin cerebrum; *Cblm.*, cerebellum; *F. cer.*, falx cerebri; *G.*, cartilage covered glandular mass; *S.*, eye-socket.

lens tissue was found. A chamber of considerable depth ( $1\frac{1}{2}$  mm.) was present in the midst of the eye-muscle mass at its point of attachment to the skin.

*Brain.* A cut was made through the upper third of the parietals and the fused frontals and the brain was exposed. The dura was very thin and closely apposed to the cranium except at two points, one being the location of the rudimentary falx cerebri and the other, the somewhat thickened tentorium cerebelli. When first viewing the brain in situ (fig. 6), one was immediately struck with the thinness of the cerebrum.

the cartilaginous mass extending from the thalamic region down over the cerebrum to the sides of the eye-socket, and the peculiar cup-shaped form of the dorsally extending cerebellum. The pia and the arachnoid closely invested the brain structures present. Although some arachnoid was closely apposed to the dura, it was judged that a distension of the subdural space by escaped cerebro-spinal fluid had taken place.

*Lateral view of the head.* Figure 7, a diagrammatic representation of the head of the pig, shows the relative thinness of the cerebrum, the size of the cavity dorsal to the brain, and the large size of the colliculi. The first three pairs of cerebral nerves were absent, but the others

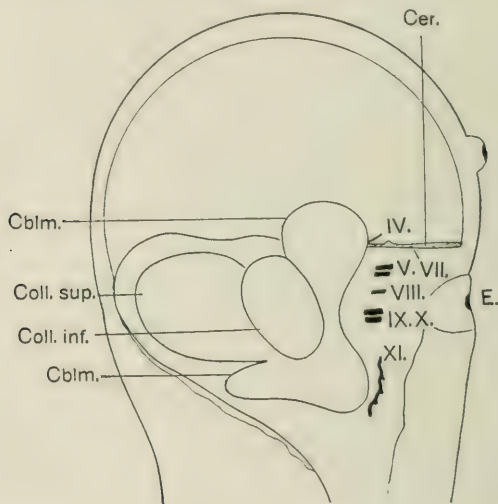


Fig. 7 Diagrammatic side view of the head of the pig. *Cblm.*, cerebellum; *Coll. sup.*, colliculus superior; *Coll. inf.*, colliculus inferior; *E.*, ear.

were present, though smaller than in the controls. A representation of the attachment of the falx cerebri has been omitted from the diagram.

*Posterior view of the brain.* In figure 8, drawn from a photograph, the thalamic mass has been elevated and the large superior and inferior colliculi are plainly seen. The third ventricle was found to be continuous with the fourth ventricle, with no noticeable constriction to form an iter. The anterior medullary velum was present and very thick; the misshapen cerebellum was relatively large.

*Mesial aspect of the brain of the pig.* Before a mesial section of the brain was made, the thin cerebrum was detached; sections were made through the cerebral tissue and particular care was taken in sectioning the cartilage in the region marked *G* in figure 6. The lump sectioned

readily and proved to consist of glands covered by a thick layer of cartilage. It is not impossible that the epiphysis was retarded in its development and forced down by a mass of overgrowing cartilage. The mesial section of the pig's brain (fig. 9) serves to show more clearly the relation between the third and fourth ventricles and also indicates the retardation of the massa intermedia. The thalamic mass was fused and the anterior tubercles were thick and extended only to a point just ventral to the epiphyseal mass.

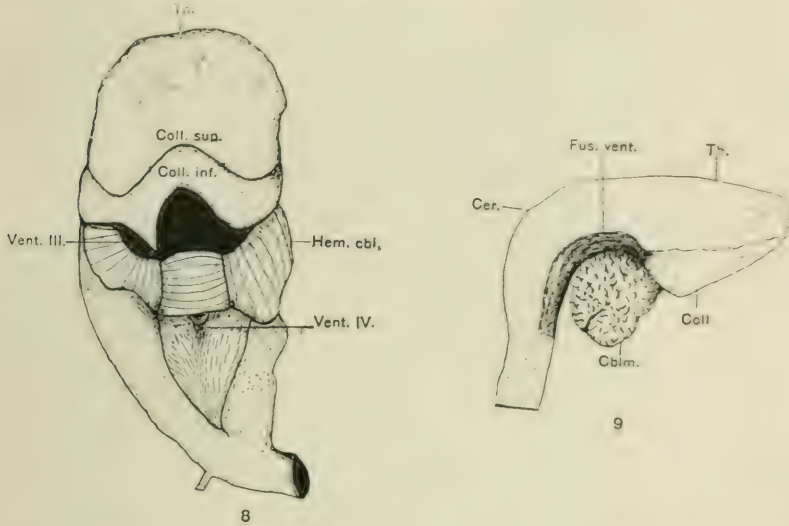


Fig. 8 Posterior view of the brain of the pig. *Coll. sup.*, colliculus superior; *Coll. inf.*, colliculus inferior; *Hem. cbl.*, Hemisphaerium cerebelli; *Th.*, thalamic mass; *Vent. III*, ventriculus tertius; *Vent. IV*, ventriculus quartus.

Fig. 9 Mesial aspect of the brain of the pig. *Cblm.*, cerebellum; *Cer.*, point at which thin cerebrum was detached; *Coll.*, colliculi; *Fus. vent.*, fused ventricles with medullary velum removed; *Th.*, thalamic mass.

*Microscopical study of sections through the cord.* The medulla and a portion of the cord were removed prior to the cutting of the mesial section and the block was embedded in paraffin; sections 15 micra thick were made and stained in hematoxylin-eosin. The cornua were well marked and the dorsal columns were particularly well seen. In an anophthalmic pig described by Duckworth ('08) the Weigert method showed the tracts as clearly as in the control. Duckworth thinks this a strong support for the theory of the laying down of the pyramidal tracts in situ. In many cases of cyclopia recorded in the literature, the descending tracts of the cord are absent. Let us briefly consider Duckworth's specimen.



*Duckworth's case of otocephalic anophthalmia.* In this specimen we find a much more extreme otocephalus than in the one which we have just noted. The pig was devoid of eyes but had ears separated. The brain was perfectly symmetrical, consisting of medulla, pons and cerebellum, with the structures connected with those parts. The cerebellum capped the pons in a manner similar to the brain just described.

#### *The human cyclops*

In none of the cases of true cyclopia with a nasal proboscis, has there been found evidence of innervation of the proboscis itself. Dr. Wilder did not study the proboscis, but he made a thorough examination of the eyeball of the specimen from which I secured the brain. We may quote briefly from Wilder's description.

#### *Eyeball and eye-muscles*

"In the eyeball itself, this specimen is decidedly double. The palpebral opening is evident and oval in shape. At the exact center of the lower margin there is a single median caruncula, with a punctum lacrymale upon either side of it on each lid component. Corresponding to the shape of the palpebral opening of the eyeball is a flattened piriform organ. The relationships and positions of the irides and pupils could not be made out, but they seem to have been turned upwards so that the optical axes would strike above the palpebral opening. Within, the eyeball is incompletely divided into two compartments by a median partition that reaches from the front wall half way back. Each compartment is furnished with a well developed lens, but that of the right side is slightly smaller than the other and not perfect in shape. Back of the lens, each compartment is nearly filled with a large mass of fine connective tissue, of almost the consistency of cartilage, which bears the lens in an anterior cup-shaped depression. This is undoubtedly the vitreous humor." (Wilder '08.)

The optic nerve was single and the eye-muscles were symmetrically disposed, showing a complete bilaterality, but the amount of abnormality was not determined. It has been found by other investigators that in cyclopean mammals, the internal recti muscles are generally absent; the obliques may be present and the external recti are generally present.

*Human brain (figs. 10 and 11).* The brain was about one-fifth smaller than the control, measuring 5.25 cm. across the widest part of the cerebrum and 7 cm. in length, measured from the most anterior part of the cerebrum to the most posterior part of the cerebellum. The cerebrum was practically smooth, and the membranes were tightly adherent to the single vesicle. There was no falx cerebri, but the tentorium cerebelli was present. The left side of the cerebral vesicle projected slightly farther posteriorly than the right side. The cere-

brum did not overtop the cerebellum but was contiguous to it at the elongation of the cerebrum on the left side. The single cerebral vesicle was thin, varying from 5 to 10 mm. in thickness, and was hydrocephalic. The cavity was formed by the fusion of the fifth and lateral ventricles. Into the cavity through the posterior opening, 3.5 mm. in diameter, projected the anterior tubercle of the fused optic thalami. The chorioid plexuses of the lateral ventricles were well developed but displaced

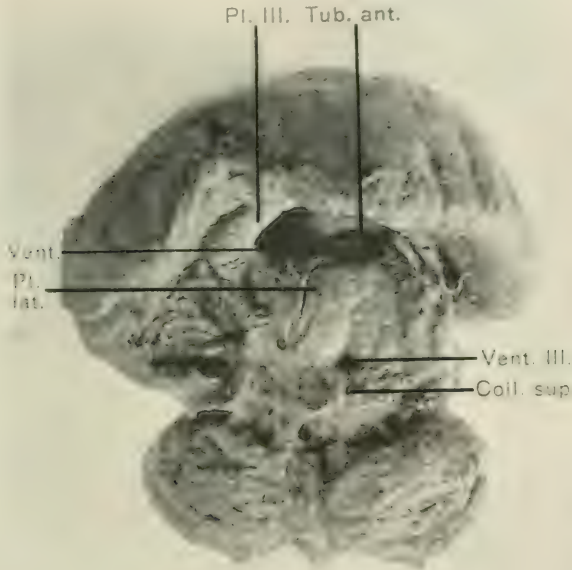


Fig. 10 Dorsal view of the brain of a human cyclops with hour-glass eye, showing the cavity of the cerebral vesicle. *Pl. lat.*, plexus chorioideus ventriculi lateralis; *Pl. III.*, plexus chorioideus ventriculi tertii; *Coll. sup.*, colliculus superior; *Tub. ant.*, tuberculum anterius thalami; *Vent.*, cavity of the cerebral vesicle; *Vent. III.*, blind third ventricle.

backwards. The lateral plexuses lay along the margin of the cavity. The fourth ventricle was covered by the thick anterior medullary velum, and the chorioid plexus of the fourth ventricle seemed well developed. The cerebellar peduncles were normal.

*Ventral aspect (fig. 11).* At the anterior end of the cerebral vesicle two depressions faintly marked it into three lobes on the ventral side. The hypophysis was present and the mammillary bodies appeared.

partly fused. The cerebral peduncles were rather large; the optic thalami were present and of normal size, but the geniculate bodies were absent. The pons, the medulla and the crura were present and well formed. The large single optic nerve was found but there was no indication of an olfactory. All the other cerebral nerves except the

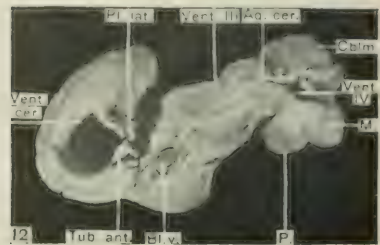
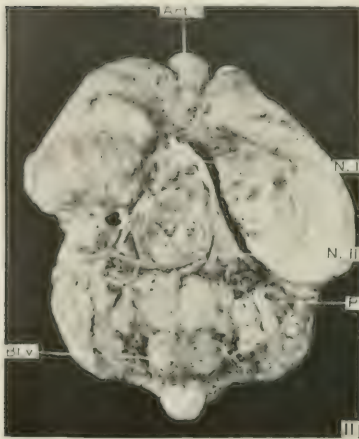


Fig. 11. Ventral view of the human brain. *Ant. l.*, anterior lobe of the cerebral vesicle; *Bl. v.*, blood vessel; *N. II*, nervus opticus; *N. III*, nervus oculomotorius; *P.*, pons.

Fig. 12 Mesial aspect of the brain of the human cyclops. *Aq. cer.*, aqueductus cerebri; *Bl. v.*, blood vessel; *Cblm.*, cerebellum; *M.*, medulla; *P.*, pons; *Pl. lat.*, plexus chorioideus ventriculi lateralis; *Pl. III*, plexus chorioideus ventriculi tertius; *Tub. ant.*, tuberculum anterius thalami; *Vent. cer.*, cavity of the cerebral vesicle; *Vent. III*, aperture of the blind third ventricle; note that the aqueductus cerebri does not connect with the third ventricle; *Vent. IV*, ventriculus quartus.

eleventh were traced. There is no reason to suppose that the eleventh was not present.

*Median section (fig. 12).* A mesial section of the human cyclops brain served to show the separation of the aqueductus cerebri from the third ventricle, and the absence of fornix, callosum and epiphysis. The fourth ventricle was apparently normal; the olivary bodies seemed well developed; the colliculi were normal in size and appearance.



## SUMMARY

This paper is based on the study of three cases of mammalian cyclopia, the first being a case of anophthalmia cyclopica in the gray rat; the second a case of anophthalmia cyclopica with otocephalus, in the pig; and the third a case of cyclopia with hour-glass eye, in man.

The rat had no external or internal indications of an eye; the pig had no eyeball nor lens, but had three lids, the two upper ones being fused almost completely; the human fetus, the brain of which was studied by the writer, had a typical hour-glass eye with two well-formed lenses. Neither the pig nor the rat had a proboscis, while the human cyclops had a well-developed single proboscis above the fused eyes.

The brains of the specimens fall readily into a series, with the brain of the rat the lowest in the scale, the brain of the pig next and the human brain the highest in development. The brain of the rat was not hydrocephalic and the cerebrum was represented by three vesicles. The cerebellum was ventral to the pons. The hypophysis was present, the epiphysis was absent and the fourth ventricle normal, no corpus callosum was present.

The brain of the pig was markedly hydrocephalic, the hydrocephalus being external and the cerebrum reduced to a thin sheet of tissue. The pons and medulla were normal, while the cerebellum formed a cup for the rest of the brain. The colliculi were normal in size and position and the brain was bilaterally symmetrical except in the posterior region of the pons. Between the anterior end of the pons and the back of the eye-socket extended two cartilaginous masses, interpreted as displaced nasal cartilages, which neither grew to form a snout nor stimulated the growth of a proboscis.

The human brain was a case of internal hydrocephalus in which the cerebrum was developed rather more than in the case of the pig. The cerebral mass was faintly marked into three lobes. The hypophysis was present and the mammillary bodies were partly fused. The pons and medulla were of normal size and configuration. A single optic nerve was present. The anterior tubercles of the optic thalami projected into the cavity of the cerebral vesicle.

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## TWINS IN FISH, ONE WITH A CYCLOPIC DEFORMITY

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FOUR FIGURES

Many cases of twins and double monsters in fish have been recorded but no case of apparent modification of structure by chemical means in one of twin fishes has been mentioned. In this paper it is my purpose to record a case of cyclopic deformity in one of twin *Funduli*, a case of double monster in *Fundulus* and a case of twins in *Squalus acanthias*.

During the summer of 1909, while occupying a research table from the Department of Zoology of the University of Chicago, at the Marine Biological Laboratory, I was engaged in putting up material for the study of the nervous systems of cyclopic and anophthalmic *Funduli*. In one of the experiments with ether, I chanced to secure twin *Funduli*, one of which was apparently cyclopic.

In the experiment, eggs from several females were fertilized by the sperm from one male and when the majority of the eggs were at the two-cell stage, a 3 per cent solution of ether in 100 cc. of sea-water was added to the eggs, which nearly covered the bottoms of two finger-bowls. The finger-bowls were then covered with glass plates to prevent too rapid evaporation. The extremely toxic action of the ether was so noticeable that at one time the separation of the living from the dead eggs was considered useless. However, two days after the experiment was started, the water was changed for fresh sea-water and a few of the dead eggs were removed. Three days from the beginning of the experiment the dead eggs were picked out and the remaining few were placed in fresh sea-water. The living eggs numbered 215 and the uncounted dead eggs numbered about 600. At the end of six days' time, the normal embryos were separated from the abnormal.

In the first lot, there were 2 cyclops, 1 pair of twins and 110 normal. In the second lot, there were 9 typical cyclops and 78 normal. The twin *Funduli* were most closely observed and were killed and preserved on the sixteenth day only because it was evident that they were about to die. Figures 1 and 2 show the defect that appeared in one of the specimens. The cyclops was the smaller of the two; the eye on the right side was apparently lacking. The heart-beat of the cyclops on the fifteenth day was 90 per minute, while that of the normal twin was 110. The smaller embryo was only about one-eighth smaller than the controls



at this time. Sections of the cyclopic twin showed that it was not a case of true cyclopia, but like many which appear in experiments with anesthetics (Stockard '10), it had a minute, deeply-buried lens in the position normally occupied by the optic vesicle.

In 1910 Mr. F. J. Kelly presented me with a double-headed specimen of *Fundulus*. The monster appeared in a lot of eggs numbering about 300. As in my experiment, the preponderatingly large number of



Fig. 1 Camera sketch of the twins.  $\times 8$ .

Fig. 2 Another view of the twin Funduli. (Camera sketch  $\times 8$  by C. R. Stockard).

Fig. 3 Double-headed Fundulus.  $\times 8$ .

dead eggs caused Mr. Kelly to consider not picking out the few living embryos. The eggs developed in 50 cc. of sea-water, fertilization having been delayed for half-an-hour in the case of about two dozen of the eggs (fig. 3).

In one of his earlier papers, Stockard mentions a case of incomplete diprosopus with three eyes and an additional lens (Stockard '09), but does not mention any factors other than anesthetic. Mr. Kelly's specimen showed four well-developed eyes. Sections were made and the specimen was found to be like many other double-headed monsters (Gemmill '00, '03). It possessed two mouths and throats, but only one heart, and had twin brains united at the optic lobes.



Fig. 4 Twin dogfish.  $\times \frac{1}{2}$ .

Through the kindness of Mr. George M. Gray of the Marine Biological Laboratory, I had the privilege of examining and photographing twin dogfish which he secured just before they would normally have been extruded. The specimens were males and were apparently normal, with a single yolk sac. Measurements showed that the twins were truly 'identical.'

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## ON THE VASCULARIZATION OF THE SPINAL CORD OF THE PIG

E. R. HOSKINS

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FIVE FIGURES

HISTORICAL

Until 1904 little work had been done upon the development of the blood-vessels of the spinal cord, except that of His ('86) who undertook to follow the growth of these vessels in human embryos. The observations of this author have been largely disputed by Sterzi ('04) and Evans ('09), the two writers who have done most of the work in this field.

By far the most comprehensive publication upon the development of the vessels of the spinal cord is that of Sterzi ('04). In this he discusses the development of the vessels in the five higher classes of vertebrates. As a type of the Mammalia he uses the sheep. He brings out the following points:

The blood-vessels first approach the cord at the ventro-lateral border and spread over the ventral surface, then over the lateral, and finally over the dorsal surface. Each vertebro-medullary artery as it approaches the cord divides into a ventral and a dorsal ramus, the ventral and dorsal radical arteries. The ventral radicals from either side halt at the lateral edges of the floor-plate, and each divides into a cranial and a caudal branch. These anastomose with those of adjacent segments and form two longitudinal arteries on the ventral surface of the cord, the "tractus arteriosus primitivus." Later they send out medial rami and through these become connected. Still later, alternate parts of the two tracts degenerate while other parts continue to develop. These enlarged segments are joined together through their medial rami and form a single ventral artery which Sterzi terms the tractus arteriosus ventralis, and which is the anterior spinal artery

of most authors. From the primitive tract, dorsal rami enter the substance of the cord. Each dorsal ramus forms a loop and gives rise to a vein, which courses ventrally and enters the primitive sulcus. Later other vessels extend into the cord from the lateral, and still later from the dorsal surface.

The dorsal radical arteries, where they divide, form many small longitudinal capillaries just ventral to the points of emergence of the dorsal nerve roots. From these capillaries there is formed later a longitudinal artery on either side of the cord, in this plane (*tractus arteriosus lateralis*). The vessels entering the cord are first solid and later become hollow.

Evans ('09) shows by a series of injected pigs the early development of anterior spinal artery. In his figures the mid-ventral surface of the cord is shown to be free from vessels until the embryos are 8.5 mm. in length, and the mid-posterior surface until after the pigs are between 8 and 10.5 mm. in length. He does not take up the later stages.

#### MATERIAL AND METHODS

For a study of this nature, injected embryos are indispensable, and they are best injected while living, with warm india ink diluted one-half with weak ammonia water. It is preferable to inject through the umbilical artery rather than through the umbilical vein, because the arteries are less readily ruptured and because the route between them and the vessels of the spinal cord is much more direct.

Embryos used for thin serial sections are better if they are congested instead of being injected. This congestion is accomplished as follows: The umbilical cord is tied while the embryo is yet living, thus causing an increase in the blood pressure in the aorta. One of the most direct outlets for this increased pressure is the system of segmental arteries, and through these the bloodvessels in and around the spinal cord soon become engorged. When this condition is reached, as evidenced by the increased redness of the dorsal region of the embryo, the live embryo is dropped into a fixing fluid which penetrates rapidly so that the capillaries are fixed before they collapse. Bouin's picro-formo-acetic mix-

ture serves this purpose very well. Embryos treated in this manner show the smaller vessels much more plainly than those fixed in the usual way.

Small injected embryos which have been cleared in oil may be dissected under the binocular microscope and all the external vessels of the cord demonstrated, or they may be sectioned in celloidin to show the internal vessels.

From pigs larger than 25 or 30 mm. the cord with its membranes may be dissected out and embedded in celloidin and cleared, or for temporary preparations may be cleared directly.

Serial sections of the cleared embryo or spinal cord can be kept permanently between two pieces of paper soaked in oil, or can be transferred to slides and mounted in damar gum. To make slide preparations, the sections are cut in oil and placed on a piece of thin paper in the same order they are to have on the slide. Another piece of oiled paper is laid down upon them and the whole inverted. The first paper is now peeled off and the other paper holding the sections is inverted upon the slide. This paper is then peeled off, leaving the sections on the slide in their proper order. They may then be washed carefully with xylol, and covered.

There are several advantages in a study of this kind, in sectioning celloidin-embedded embryos free-hand in oil. The cord can be turned so that sections may be cut through any plane. Sectioning is done more rapidly than with a microtome, and much time is saved. It is easy to make transverse, sagittal and frontal sections from any region of the same cord. The block can be examined with a lens, during the sectioning, and any particular vessel or vessels included in a section. Also, sections may be made of different shapes.

#### BLOOD-VESSELS OF THE NEAR FULL-TERM FETUS

In order to determine as near as possible the arrangement of the blood-vessels of the spinal cord in the adult condition, a number of fetal pigs, near full-term, were injected and their spinal cords dissected out for study. Although these showed some variation in the blood-vessels, as is to be expected, a general



plan was to be observed. The following description is of the typical condition found in the blood-vessels in and around the spinal cord of pigs of about 240 mm. in length:

The terminology used by Kadyi ('89) for the blood-vessels of the adult human cord will be referred to frequently.

On the surface of the cord at this stage are four main longitudinal arterial systems which are located, one, median on the ventral surface; one, on each dorso-lateral surface; and one median on the dorsal surface.

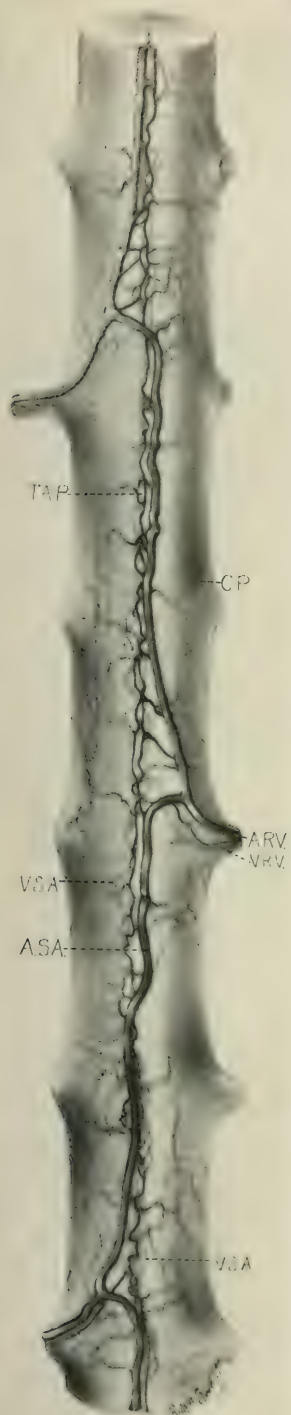
The vertebro-medullary branches of the dorsal segmental arteries approach the cord laterally and each divides into a dorsal and a ventral branch termed the posterior and ventral radical arteries respectively (figs. 1 and 2). The latter reach the cord along the cranial surface of the spinal nerves and ganglia, and, on the cord, run cranially. They may be equally distributed to the two sides of the cord and to the different regions of it, or some regions may have more than others. They average eighteen in number. The ventral and dorsal radical arteries have lost their connection with the vertebro-medullary artery in places and extend between the artery on the ventral surface of the cord and one of those on the dorso-lateral surfaces.

Soon after reaching the cord each ventral radical artery branches, giving off one ramus which courses cranially, and another which extends caudally. These divisions anastomose with those of neighboring ventral radicals, on the same or opposite side

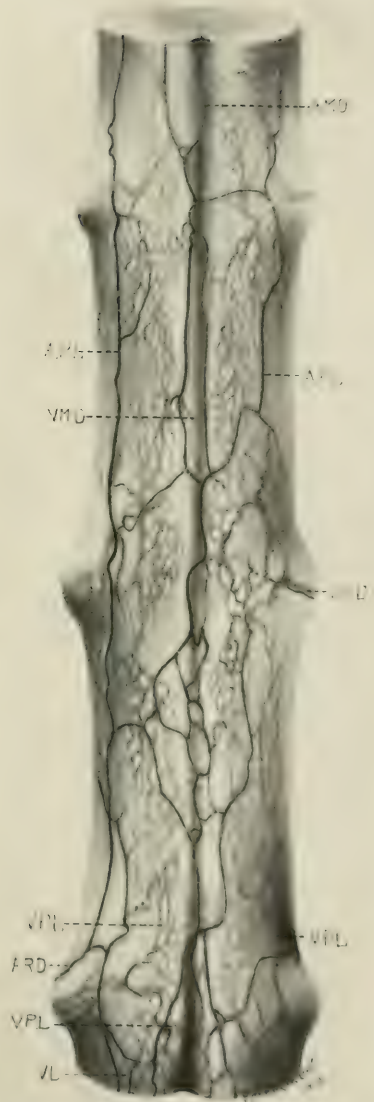
Fig. 1<sup>1</sup> Ventral surface of the middle thoracic region of the spinal cord of a fetal pig, 240 mm. in length. *A.S.A.*, anterior spinal artery; *A.R.V.*, ventral radical artery; *T.A.P.*, accessory anterior spinal artery (remains of primitive arterial tract); *V.R.V.*, ventral radical vein; *V.S.A.*, anterior spinal vein; *C.P.*, capillary plexus.  $\times 6$ .

Fig. 2 Dorsal surface of the same part of the cord shown in figure 1. *A.M.D.*, median dorsal artery; *A.P.L.*, dorso-lateral artery; *A.R.D.*, dorsal radical artery; *V.M.D.*, median dorsal vein; *V.P.L.*, dorso-lateral vein and plexus; *V.R.D.*, dorsal radical vein; *V.L.*, dorso-lateral venous plexus.  $\times 10$ .

<sup>1</sup> The figures in this paper, except numbers 3 and 4, were drawn with a camera lucida. The size of the embryos is the greatest length, as measured in 75 per cent alcohol.



1



2

of the cord, and form in this way the anterior spinal artery, which lies in or near the median ventral line. Occasionally a radical artery, instead of dividing, goes across the cord and joins the cranial or caudal ramus of the one on the opposite side. The anterior spinal artery has a winding course, bending laterally to meet the vessels which form it, and making many smaller irregular bends to one side or the other. In some places it may lie to one side of the mid-line for several segments and where this occurs there are found numerous longitudinal rami of the anterior spinal artery, or the cranial and caudal divisions of the ventral radicals. These may be called accessory anterior spinal arteries, and are sometimes present even where the anterior spinal artery lies in the mid-line. Here they are located between this vessel and cord, at the lip of the ventral median fissure. They are the remnants of the "tractus arteriosus primitivus" of Sterzi.

The dorsal radicular rami of the vertebro-medullary arteries are much more numerous than the ventral ones. They course dorsally along the cord and in a slightly cranial direction, to a plane just ventral to the emergence of the dorsal roots of the spinal nerves, where they divide into two rami, one extending cranially and one caudally. Each of these rami anastomoses with the one of the adjacent segment, and thus there is formed on either side an irregular longitudinal vessel, the dorso-lateral artery (fig. 2; tractus arteriosus postero-lateralis of Kadyi). From this artery recurrent rami supply the dorsal nerve roots and spinal ganglia, and the lateral surface of the cord. Other rami, two or three in each segment, and much larger than the above, run dorsally and by longitudinal anastomoses with each other, and with similar rami from the opposite side, form an artery in the mid-dorsal line which may be termed the median dorsal artery.

Very small rami from the dorso-lateral arteries run ventrally along the cord and unite with others from the anterior spinal artery, forming a plexus on the ventral and lateral sides. These ventral rami of the dorso-laterals anastomose freely in a longitudinal direction and form one or more small longitudinal arteries between the ventral and dorsal nerve roots in some parts of the cord. These are the tracti arteriosi laterales, and ventro-later-



ales, of Kadyi. Still other small rami from the dorso-lateral and median dorsal arteries form a capillary plexus on the dorsal and dorso-lateral surfaces of the cord. In a few places along the cord the dorso-lateral arteries are double, one division lying dorsal to the dorsal nerve roots, and corresponding perhaps to the "tractus arteriosus posterior" of Kadyi.

The median dorsal artery is a very irregular longitudinal vessel formed by the dorsal rami of the dorso-lateral arteries, as described above. In places it is double or may show a longitudinal capillary arrangement. Many of its lateral rami anastomose longitudinally forming small arteries parallel with the median dorsal artery (fig. 2). This is also true of the dorsal rami of the dorso-laterals. By the anastomoses of the rami of the various arteries just described, the entire cord is surrounded by an arterial vascular system, and from all parts of this network smaller arteries penetrate its substance.

The veins of the spinal cord are in three principal longitudinal systems, and other smaller ones. Of the three, two are dorsal and one ventral. All three show evidence of their capillary origin. The anterior spinal vein is the smallest of the three (fig. 1). It lies between the cord and the arteries, in the median ventral line. It is larger than the accessory anterior spinal arteries, but never attains the size of the anterior spinal artery proper. It is very irregular and in some regions is entirely replaced by a narrow network of capillaries.

On either side of the median ventral sulcus, the cord is covered with large venules, some of which lie between the cord and the arteries, and some of which are external to the latter. They are often two or three times as large as the arterioles to which they correspond. They anastomose freely with the anterior spinal vein and empty laterally into the ventral radical veins which are in close relation with the ventral nerve roots and ventral radical arteries, but which are much more numerous than the latter, one being present on nearly every nerve root. They drain blood also from the lateral surface of the cord. Their ventral and dorsal rami often form short, small, longitudinal veins by anastomoses, some of which in other animals have been named, antero-lateral.

etc. The blood from the anterior spinal veins and venous capillaries of the general ventral surface form, in places, transverse channels which are perhaps large enough to be called veins.

On either side of the dorsal surface of the cord there extends longitudinally a large irregular vein, the dorso-lateral, about half way between the artery of the same name, and the median dorsal artery. Some parts of these vessels and their rami, like the ventral venous capillaries, lie external to the arteries and some internal to them. They are the largest vessels on the cord with the exception of the anterior spinal artery (compare figs. 1 and 2). Dorsally these veins are united through large capillaries, and blood leaving the cord in the median line may flow either to the right or left. Half way between two consecutive nerve roots the dorso-lateral veins usually break up into many divisions so that each may be seen to drain blood from adjacent halves of two segments. Laterally they empty through one or more divisions into the large dorsal radical veins, one of which lies upon each dorsal nerve root (fig. 2).

A fourth longitudinal venous system, smaller than the three described, lies in the median dorsal sulcus. It resembles the dorso-lateral veins except that it is more irregular, and in places it may be entirely lacking. Its lateral rami empty in the dorsal venous capillary plexus or directly through larger vessels into the dorso-lateral veins. It may be termed median dorsal venous system.

Some of the venous capillaries of the lateral surface drain into the dorso radical veins, some into the dorso-lateral veins, and some into ventro radical veins, and all these vessels together with the anastomoses of the veins on the ventral and dorsal surfaces already mentioned above, completely surround the cord with a venous system, corresponding to the system described for the arteries.

Of the arteries entering the cord, the largest are those in the ventral fissure, the ventral central arteries, which form two nearly parallel rows, but which are not paired. They arise from the anterior spinal arteries, or the accessory anterior spinal arteries. They show evidence of the capillary origin in longitudinal anas-

tomoses found between vessels of the same side. These anastomoses are numerous in the fissure, particularly near the vessels from which the ventral central arteries arise.

The ventral central arteries vary considerably in size, some being as large as the vessels they arise from and others much smaller (fig. 3). The course of the smaller vessels is usually more irregular than that of the larger. They pierce the substance of the cord at different levels, some entering near their origin and others extending some distance into the fissure. Their general course is dorso-lateral, but those entering near the mouth of the fissure may bend very sharply to the side and enter the ventral horn of the gray substance. The others course more dorsally nearly to the level of the central canal where they make a decided lateral bend, and divide into two or more rami, although sometimes they give off rami more ventrally than this (figs. 3 and 4). The principal divisions of these arteries extend in a longitudinal plane, and anastomose with similar rami of adjacent vessels. They also give off smaller arteries and capillaries which ramify through the gray matter in all directions, helping to form a dense plexus. The longitudinal arteries tend to form loops after they have coursed in one direction for a short distance, as they do in young embryos (fig. 5). One artery may form several such loops, producing as many longitudinal vessels, each succeeding vessel lying dorsal or lateral to the last, and of a lesser caliber. These smaller longitudinal vessels anastomose with each other ventro-dorsally and laterally by rami which usually leave them at right angles, and also anastomose with rami from vessels other than the ventral central arteries, as will be described later.

Besides the rami of the central arteries just described, other rami extend farther laterally into the gray substance before branching. Some of these, instead of forming longitudinal vessels, form small irregular ones which ramify through the gray matter in all directions, anastomosing with similar vessels from other arteries in this region and forming a dense capillary plexus in the ventral and dorsal horns.

Other arteries, smaller than the ventral central arteries, enter the cord from the dorsal median sulcus and course ventrally and



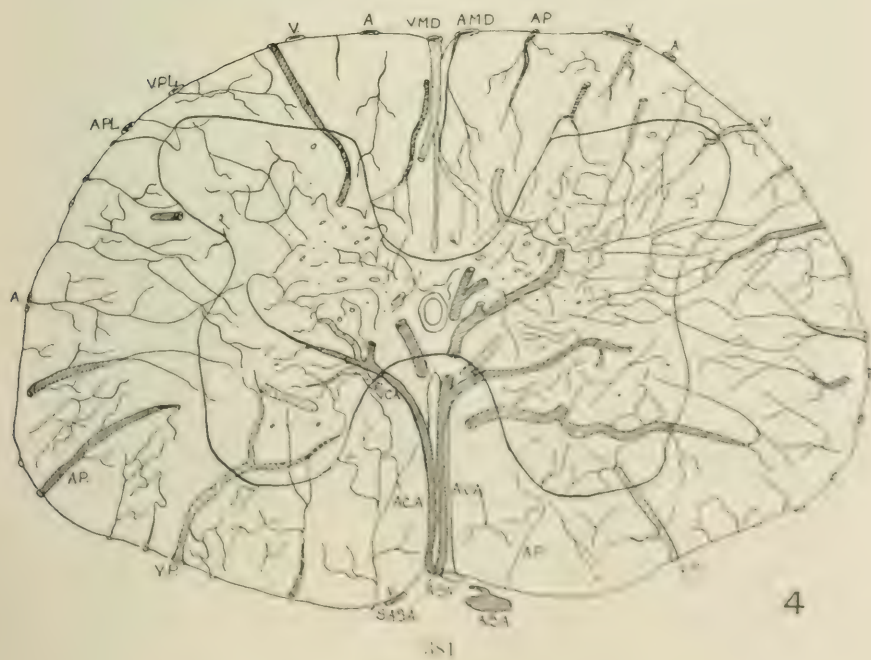
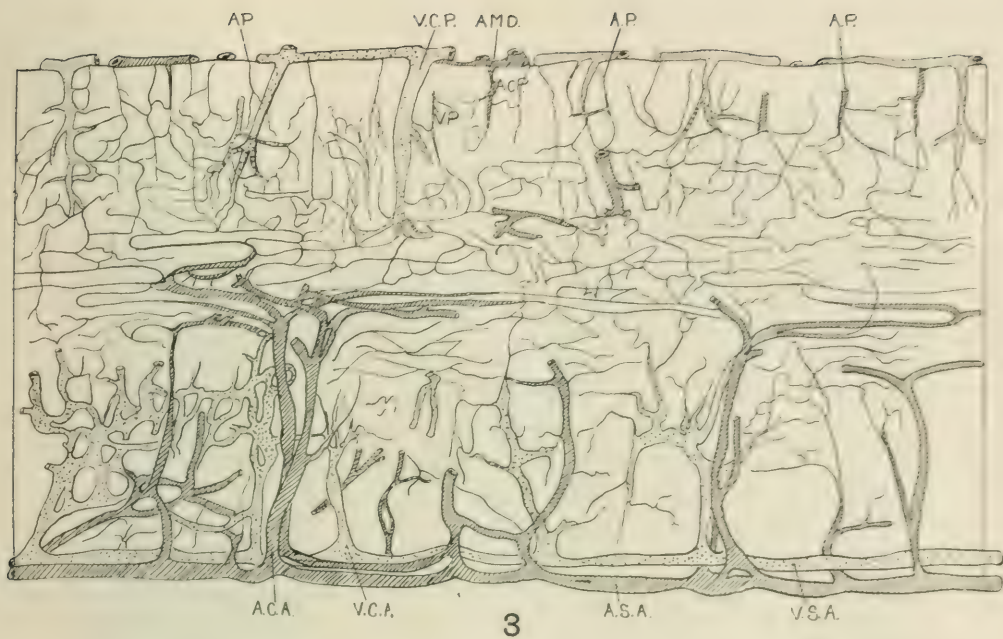
laterally to the dorsal horns of the gray substance. Here they form still smaller vessels resembling to some extent those formed by the ventral central arteries, but most of their rami are short and do not extend longitudinally. These may be called the dorsal central arteries, but they are more similar to the peripheral arteries from other surfaces of the cord, than to the ventral centrals, and perhaps should be called dorsal peripheral arteries. They give off many small lateral rami in the white substance and in the outer part of the gray substance.

In addition to these vessels, other small arteries enter the cord from all sides, from the arteries and arterioles which surround it. These are the peripheral arteries referred to above. They are very numerous and in a single thick cross section as many as fifty or sixty of them may be counted. They give off short rami in the white layer of the cord and extend into the gray layer. These rami branch and anastomose and form a loose capillary network. The vessels entering the gray substance enter into the longitudinal plexus already described and give off lateral rami which branch freely and anastomose.

The longitudinal vessels which arise from the ventral central arteries are quite large, but the other vessels formed from these trunks, and those formed from the dorsal central and peripheral arteries, are much smaller. A very thick section presents a picture of an inner core of longitudinal vessels with other vessels extending into it at right angles from all points on the periphery of the cord (figs. 3 and 4).

Fig. 3 Sagittal section from the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.C.A.*, ventral central artery; *A.P.*, peripheral artery; *A.C.P.*, dorsal central artery; *V.S.A.*, anterior spinal vein; *V.C.A.*, ventral central vein; *V.C.P.*, dorsal central vein; *V.P.*, peripheral vein.  $\times 35$ .

Fig. 4 Transverse section through the lower thoracic region of the spinal cord of a 240 mm. fetal pig. *A.S.A.*, anterior spinal artery; *A.P.*, peripheral artery; *A.*, artery; *A.C.A.*, ventral central artery; *S.A.S.A.*, accessory anterior spinal artery; *A.P.L.*, dorso-lateral artery; *A.M.D.*, median dorsal artery; *A.S.V.*, anterior spinal vein; *V.*, vein; *V.P.*, peripheral vein; *V.P.L.*, dorso-lateral vein; *V.M.D.*, median dorsal vein.  $\times 35$ .



## DEVELOPMENT OF THE BLOOD-VESSELS

The early development of the anterior spinal artery has been described by Evans ('09) and Sterzi ('04).

Some pig embryos of 12 mm. show a fairly well developed anterior spinal artery, while in others of 14 or 15 mm. it is just beginning to form. Although, after this vessel is once formed, it does not undergo marked changes, there is some modification. For example, the ventral radical arteries meet it at right angles or nearly so, until the embryo is 40 or 45 mm. in length. Thereafter, the growth of the cord and the fixed position of the radicals seem to cause the artery to be pulled laterally by the radicals, and a gradually decreasing angle is formed at the points where the radicals meet it (fig. 1).

As the embryo grows, the number of radical arteries continues to decrease even after the anterior spinal artery is well formed. This seems to be true until the embryo reaches the length of about 100 mm.

Some of the arterial capillaries on the ventral surface of the spinal cord are continuous with the anterior spinal artery directly, or indirectly through remains of the tractus arteriosus primitivus, and others are continuous with the capillaries of the lateral surfaces of the cord.

A dorso-lateral artery is formed in the capillary plexus on each of the lateral surfaces of the cord, just ventral to the point of emergence of the dorsal nerve roots. The dorsal radical arteries branch in this region and give off dorsal and lateral rami, which are continuous with the lateral capillaries just mentioned. A very irregular longitudinal vessel develops where certain of these

Fig. 5 Transverse section through the mid thoracic region of the spinal cord of an 11 mm. pig embryo. *T.A.P.*, primitive arterial tract; *R.D.T.A.P.*, *R.L.T.A.P.*, *R.M.*, dorsal, lateral, and medial rami of the primitive arterial tract; *A.V.M.*, vertebro-medullary artery; *A.R.V.*, *A.R.D.*, ventral and dorsal radical arteries; *C.R.*, *Cr.R.*, *D.R.*, *V.R.*, caudal, cranial, dorsal and ventral rami of the dorsal radical artery; *D.P.*, *D.L.P.*, dorsal and dorso-lateral capillary plexuses; *D.L.C.G.*, *V.L.C.G.*, dorso-lateral and ventro-lateral groups of peripheral capillaries; *V.L.V.P.*, ventro-lateral venous plexus; *V.R.V.*, *V.R.D.*, ventral and dorsal radical veins; *S.P.G.*, spinal ganglion; *V.N.R.*, *D.N.R.*, ventral and dorsal nerve roots; *S.N.*, spinal nerve.  $\times 200$





capillaries increase in size, perhaps on account of the increased pressure from the dorsal radical arteries. This longitudinal vessel is indicated in embryos of 12 mm. and is quite strongly developed in embryos of 15 to 18 mm. In these stages it seems to dip ventrally to meet the approaching radicals, as pointed out by Sterzi for the sheep ('04). As the embryo grows, this dorso-lateral artery becomes more and more regular. It is still somewhat irregular in embryos of 60 mm. but quite regular in those of 75 mm. The dorso-lateral artery never attains the size of the anterior spinal nor is it ever so regular in its course. In places it may develop as two or more vessels, but these are always smaller than the single artery. The dorso-lateral arteries are each continuous with the capillaries of half the cord in the early stages, but as the cord increases in size they supply directly only the dorso-lateral surface.

The capillary network on the lateral surface of the cord is at first continuous with that extending through the mesenchyma of this region as far, laterally and dorsally, as the myotomes and body wall respectively. In later stages when the membranes of the cord begin to develop, the connections between the vessels of the cord and those in the mesenchyma around it are lost.

The median dorsal artery is the last of all the vessels on the cord to develop. In pigs of 30 mm. it is still very irregular and indefinite, and is entirely lacking in places, although the vessels which go to form it, the dorsal rami of the dorso-lateral arteries, may be seen in embryos of 20 mm. In pigs of 45 mm. it is quite definite, lying in or near the mid line of the dorsal surface, as described above for the 240 mm. embryo. It never becomes very regular, and in pigs of 100 mm. it resembles the condition seen in the pig of 240 mm. It is continuous with the arterial capillaries of the dorsal surface of the cord and with the dorso-lateral arteries.

In addition to these main arterial trunks there develop on various parts of the cord, especially on the lateral surfaces, short longitudinal arteries. These are never large or regular. They have been described in connection with adult human cord under the terms "tractus arteriosus; ventro-lateralis, posterioris, and

lateralis" (Kadyi). Of these, the "tractus arteriosus posterior" is the most prominent and corresponds to the description in this paper of parts of the dorso-lateral artery, where it sometimes has two divisions, one of which runs dorsal to the dorsal nerve roots and the other ventral to them. The dorsal divisions are evidently the same as this 'tractus.'

The veins on the cord develop in much the same way as do the arteries. The ventro-lateral surface of the cord in very young embryos is covered with capillaries, and these are continuous laterally with the capillaries in the mesenchyma round the neural tube. Medially they become continuous with the lateral rami of the primitive arterial tract. When this tract becomes separated from the cord by the ingrowth of mesenchyma, these capillaries send medial outgrowths between the tract and the cord, as seen in embryos of 12 to 15 mm. Dorsally they grow along the cord and spread over the dorso-lateral surface (pigs of 6.2 mm.) and later over the dorsal surface (pigs of 7.5 mm.). Laterally they spread over the ganglia.

From the ventral surface, the blood draining away through the capillaries soon establishes segmental vessels, the ventral radical veins, which course laterally along the nerve roots. Each radical vein on one side drains adjacent halves of two segments. These receive blood from the capillaries of the ventral, lateral, and ventro-lateral surfaces. Lying in the ventral median fissure in young embryos, small longitudinal veins may be seen in different regions of the cord, and in embryos of 25 to 30 mm. a fairly definite longitudinal vessel may be found here. This vessel in still older embryos becomes a more definite trunk and may be called the anterior spinal vein. It never attains the size of the anterior spinal artery. Laterally it drains into the ventral radical veins.

Some of the ventral and lateral capillaries of the younger embryos, early become differentiated into veins. This is especially true of the dorsal vessels. From these, some of the blood drains laterally out through vessels in the mesenchyma to the myotomes. A pig of 6.2 mm. shows three planes in which this occurs, one on a level with the dorsal surface, one just above the level of the ven-



tral surface, and one about half way between the other two. At the myotomes the blood drains ventrally into the intersegmental veins. Some of the capillaries of the lowest of these three planes, which drain the blood from the lateral surface of the cord and from the ganglia, soon become large and are called the vertebro-medullary veins, one pair of which is formed for each segment. In older embryos they course along the spinal nerves with the vertebro-medullary arteries. They receive the blood from the ventral and dorsal radical veins. The former have been described. The latter develop along the sides of the ganglia in the capillaries already mentioned. At first they carry only a part of the blood from the dorsal surface of the cord, but later (pigs of 25 mm.) they carry practically all of it. They are more numerous than the corresponding ventral radicals, and are found in every segment.

The venous capillaries of the dorsal-lateral surface on either side draining toward the nerve roots early establish longitudinal veins. These are only about half as long as a segment of the cord. Figure 5 of an 11 mm. pig, shows an indifferent plexus on this surface, but in 15 to 17 mm. embryos, fairly definite vessels may be seen. These become more and more regular as the animal develops, and as embryos of 50 to 60 mm. show, they form a venous system on either side of the cord just dorsal to the dorsal nerve roots, much like that described for the 240 mm. stage. These systems constitute the dorso-lateral veins (fig. 2).

The first blood vessels entering the cord grow in as capillaries from the ventral surface. Sterzi ('04) reports vessels in the cord of a sheep of 5.5 mm., but they were not apparent in the cord of pig embryos of less than 7.5 mm. These vessels are the dorsal rami of the primitive arterial tracts, of the lateral rami of these tracts, and of the other capillaries near the median line. They are the first indications of the central arteries and veins. They form two nearly parallel rows, one on either side of the ependymal layer, or some of them may lie in this layer. They grow dorsally about half way to the dorsal surface of the cord. They exhibit numerous longitudinal anastomoses and form a plexus along the lateral side of the ependymal layer in each half of the cord. These are true capillaries at first, but soon differentiate into arteries and veins.

Those coming directly from the primitive arterial tracts all become arteries, while those coming from the vessels lateral to the tract may become either veins or arteries.

In embryos of 9.5 mm. another group of capillaries may be seen to have entered the cord. These come from the lateral surface, extending medially nearly to the central canal. Later they anastomose ventro-dorsally and longitudinally, among themselves and with the vascular sprouts from the ventral surface.

The vessels in the cord of a pig of 11 mm. present the following characteristics, as shown in figure 5. Rami from the primitive arterial tract may anastomose with those from the ventral capillaries. Neighboring vessels of the same kind anastomose freely and give off lateral rami into the anlagen of the ventral horns of gray substance. These rami branch and anastomose with each other and form loops which anastomose with the central vessels from which they arise, or with neighboring vessels. In a plane just above the anlagen of the ventral horns each of the central vessels ends blindly, or divides into a caudal and a cranial ramus, which anastomose with adjacent similar rami and form irregular longitudinal vessels. By other anastomoses among the central vessels, a longitudinal plexus is formed, which covers very completely the lower half of the lateral side of the ependymal layer.

A comparison of figures 3 and 5 shows how closely the form and arrangement of these capillaries corresponds to that of the future central arteries and veins. Besides these main capillaries two smaller lateral groups are present at this stage. These may be called the ventro- and dorso-lateral groups, and later form peripheral arteries and veins. Both groups enter the cord from the capillaries on the lateral surface between the dorsal and ventral nerve roots. The ventro-lateral group enters at the level of the dorsal extremities of the central vessels, and courses medially and anastomoses with them. Occasionally the ventro-lateral group gives off rami which extend into the anlagen of the ventral horns. The capillaries of the dorso-lateral group are confined to the dorsal two-fifths of the cord, and although they anastomose with each other at this stage, they do not anastomose with the central or ventro-lateral capillaries. They course medially and

dorsally along the ependyma, ending blindly or forming loops, but do not reach the dorsal surface.

As development proceeds, the lateral groups of capillaries shown in figure 5 spread dorsally and ventrally and capillaries enter the cord from the periphery. With the exception of the above-mentioned dorso-lateral group of capillaries, all the vessels entering the sides of the cord grow toward a common center, namely, an area on the lateral border of the ependyma about half way between the dorsal and ventral surfaces. The dorso-lateral group of capillaries which are shown in the same figure send rami toward this center after the embryo attains the length of 14 mm.

The vessels from the dorsal surface grow ventrally along the ependyma and unite with the dorsal rami of the primitive arterial tract. This union continues the plexus on the lower part of the ependyma dorsally so that the ependyma except below the floor-plate and above the roof-plate, is entirely surrounded by a capillary plexus. A thick transverse section of the cord of an embryo of 25 mm. shows this plexus with numerous vessels extending from it laterally at right angles. These lateral vessels are joined together by dorso-ventral rami. This picture is characteristic of the cord until the embryo reaches the length of 30 or 35 mm. when it is changed by other peripheral vessels meeting the ependymal plexus obliquely and by the branching of the vessels in the anlage of the gray substance.

By this time the central arteries from both the ventral surface (ventral central arteries) and from the dorsal surface (dorsal central or dorsal peripheral arteries) have become quite large, although the latter do not nearly equal the size of the former. The ventral central arteries have formed more longitudinal loops similar to those shown in figure 5. They are separated more and more from each other, owing to the growth of the cord, and as this separation continues the longitudinal vessels grow in length.

In embryos of 35 to 40 mm. in length the peripheral arteries from all sides together with the lateral rami of the central arteries have formed a dense plexus in the gray substance, although the white substance contains only the peripheral arteries running through it, and the short branching rami given off at right angles



from them. By the time the embryo reaches a length of 50 mm. the capillaries in the white layer have much the same appearance as those of the full term fetus, except that in the latter they branch and anastomose more freely and the growth of the cord tends to separate both the peripheral vessels and the central vessels. Embryos of 75 to 100 mm. in length show the arteries in the cord quite as completely developed as in the 240 mm. embryo.

The posterior rami of the primitive arterial tract in the ventral part of the cord of embryos of 12 to 15 mm. are more numerous than the central arteries in the 240 mm. embryo which are formed from them.

The veins within the cord develop in the same planes as the arteries, and from the same plexus of capillaries that form the latter. They may be called the central and peripheral veins corresponding to the similarly named arteries. They are shown in figures 3 and 4 in a fully developed condition.

#### SUMMARY

The dorsal rami of the primitive arterial tract, and other rami from the capillaries in its immediate vicinity enter the cord, forming an undifferentiated capillary plexus (fig. 5) and this plexus later becomes differentiated into arteries and veins. It was not found, as stated by Sterzi for the sheep, that each dorsal ramus of the primitive arterial tract grows into the cord, and forms a loop, giving rise to a vein which grows back along the artery to the ventral surface.

The dorsal rami of the primitive arterial tract are more numerous than the ventral central arteries which develop from them.

Sterzi reports solid blood-vessels in the cord of sheep of 5.5 mm. and hollow ones in those of 6.6 mm. In pig embryos the blood-vessels within the cord seemed to appear first as hollow vessels. These are seen first in embryos of 7.5 mm. in length.

The "tracti arteriosi laterales" of Sterzi, are the dorso-lateral arteries of this and postero-lateral of other papers, and are the posterior spinal arteries of human descriptive anatomy. Evans shows these two tracts first united by medial anastomoses in a

pig of 8.5 mm. in length, but many such anastomoses are to be found in embryos as small as 7.5 mm. in the cervical and thoracic regions, and one specimen of 6.2 mm. showed them in the cervical region.

The embryos described in this paper show the mid-ventral and mid-dorsal surfaces of the cord to be covered with blood-vessels at a somewhat earlier stage than has been described.

As reported by Sterzi ('04) and Evans ('09), blood-vessels first appear on the ventro-lateral surface of the cord, then on the ventral, then on the dorso-lateral, and finally on the dorsal surface.

The blood-vessels on the cord are continuous with those in the mesenchyma surrounding it until the membranes of the cord are formed.

It is generally stated in textbooks of human anatomy that the spinal artery arises from the vertebral arteries, and is reinforced by segmental spinal arteries. It is rather to be considered that this artery arises from the segmental spinal arteries, and anastomoses with, or is reinforced by, the vertebrals.

The term median dorsal is suggested for the artery present in places in the median dorsal line of the spinal cord.

My thanks are due to Dr. Richard E. Scammon for his constant interest in the progress of this work, and for his many helpful criticisms.

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## A COURSE OF CORRELATIONAL ANATOMY

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During the first year and a half of study the medical student acquires a knowledge of certain aspects of the structure and function of the human body. Since these different aspects are studied in separate courses the tendency is for the student to acquire certain disjointed groups of facts which neither his ability nor the time at his disposal permit him unassisted to bring together. Such a mass of fragmentary knowledge falls far short of what the student is supposed to have acquired, namely, a reasonably good understanding of the structure and activities of the entire organism. It is true that in every good course the instructor brings the subject matter of his speciality into relation with that of other courses; this correlation in the separate courses is indispensable and serves as a foundation for further efforts in this direction, efforts made by the student alone, or preferably under suitable supervision. But in attempting in each course to correlate the subject matter with that of other courses the results obtained are inadequate, not only on account of the lack of sufficient time but especially because the knowledge of the student is as yet too limited. It therefore appears advisable, after the various courses involved have been completed, to renew in a special course the effort to help the student bring together certain important facts already learned piecemeal. This article deals with such a course introduced this year by the Department of Anatomy of the University of Cincinnati.

The course in correlational anatomy is the logical result of the method of instruction in the Department of Anatomy. The head of the department, Professor Knowler, has in all the work of the department constantly insisted upon the correlation of

structure and function. And accordingly those portions of the body which are of the greatest functional importance have in the courses of the department received the greatest attention. A further advance consists in the increasing correlation between the different courses of the department (gross anatomy, histology, neurology and regional anatomy). The work in gross anatomy and in histology is so related that the student studies the gross and microscopic anatomy of each organ simultaneously, as far as this is practicable; this is only one instance of the correlation of work within the department. In the second year the association of gross anatomy, histology, physiology, neurology and regional anatomy is much closer. This intimate relation between the separate courses within the department is maintained by the close association of the various members of the staff so that each member is familiar with the nature and progress of each course; this knowledge is attained not only by means of frequent conferences but also through actual experience in assisting from time to time in each course. On the other hand, such a correlation of courses within each department makes additional demands upon the staff, demands which are difficult to meet unless the department be supported in a more liberal manner than is customary. It is manifestly unreasonable to blame the student for regarding different courses as unrelated when the instructors themselves behave as if this were true, and it is also unreasonable to expect instructors to establish such a correlation if they be already overworked. It is upon this intimate association of the different courses within the department that the course in correlational anatomy is founded. While the author has designed this course and has conducted it alone, he does not claim the entire credit of originating it, since it is the result of the whole attitude which Professor Knowler has impressed upon the department.

In addition to correlating its own courses and introducing a considerable amount of general biology, embryology and physiology, the Department of Anatomy has repeatedly requested other departments to send students back for supplementary study whenever these departments demand special anatomical knowl-



edge which the student cannot and should not obtain during his regular courses in anatomy. Such supplementary work has proved of great advantage to the students, since it is undertaken by trained men who realize the need of the knowledge which they return to acquire, and they gladly accept the opportunity. The advantages which an anatomical laboratory possesses as against didactic teaching are evident. This arrangement is impossible if the student's time be completely occupied by required work; in addition it makes demands upon the time of the anatomical staff, and the question arises as to whether the institution is willing to pay for such advantages to the students or whether it will take the course of least resistance and of least expense.

The course in correlational anatomy is given at the beginning of the second semester of the second year. Before it begins the student has finished dissecting the body and has completed, beside other courses, those in histology, physiology and neurology; in addition, he has studied the various aspects of anatomy from a physiological standpoint. During this year and a half the student has amassed a large number of facts and has made some progress in bringing isolated facts together; moreover, he has attained to a considerable degree the ability to recognize essentials and to work problems out for himself. The course in correlational anatomy lasts eight weeks, and during the remainder of the second semester the student is at liberty to elect such work in the department as he may desire; he may take the course in regional anatomy, or he may spend the time reviewing the essentials of the body, working out for himself (with the assistance of the staff) certain important mechanisms not given in the course in correlational anatomy. This course is accordingly preceded by the study of the structure and function of the entire body, and is followed by a period during which the student has the opportunity of bringing together on his own initiative further disconnected groups of facts; in this manner the student is encouraged to form the habit of study outlined in the course just completed so that he may thus acquire a real knowledge of the human body. This result is aided by the fact that at the end of the second year he

must pass an examination which includes all the subjects studied in the department and in which a fair but real knowledge of the essentials of the body is demanded.

At this point the author would like to enter a protest against the unfortunate tendency in some institutions to complete the work in anatomy during the first year. The student can undoubtedly finish the courses in histology and neurology and dissect the entire body in one year, but even if the time should be sufficient to permit the student to finish his task without undue haste the result would still be unsatisfactory. For a real knowledge of anatomy cannot be acquired all at once, but only when the study is prolonged to such an extent that the student has the repeated opportunity of thinking of the problems which the dissection of the body merely makes possible to study. Moreover, the student's knowledge of physiology and his capacity for independent constructive thinking is too limited to permit him to obtain an adequate knowledge of the body during the first year. Finally, this excessive concentration and hurry encourages the student to regard each of the anatomical courses (and each part of each course) as a separate task to be gotten out of the way in a definite length of time, and not as aspects of one great problem to be correlated with one another and with those aspects learned in other departments.

The course in correlational anatomy consists in the study of certain mechanisms of the body. New matter is introduced only when necessary, while on the other hand, unessential details are eliminated. The course therefore attempts to help the student rescue from the mass of details the really vital facts concerned in each mechanism, and by correlation of these facts to fix them firmly in his memory. Especial emphasis is placed upon the relation of the nervous system to the rest of the organism, and the various reflexes involved in the activities of each mechanism are studied thoroughly, the path of the impulse being followed throughout its entire extent. The course differs from one in physiology in that the anatomical structures upon which depend the activities of any mechanism become to the student realities; as far as possible the actual anatomical structures are

studied in the gross and in sections, and in the nervous system the actual location of nervous centers and the course of fiber tracts are reviewed not only in diagrams but also in the specimens themselves.

For the most part the student is expected to work out, with the aid of specimens and books, the various problems for himself. Since all anatomical and physiological facts involved have been previously studied it is possible to assign at each exercise a large field to be covered, and to expect the student, with a certain amount of guidance, to select the most vital points and to ignore nonessentials; the value of this training is evident. While lectures are necessary they are mostly informal, assuming the character of conferences, while at the beginning of each exercise the main results of the work of the preceding day are briefly summarized. Finally, the student is expected to hand in at the end of the course a complete account of the mechanisms studied, and to describe certain activities which have not been studied but with whose anatomical basis he is supposed to be familiar.

In selecting topics for study in a course in correlational anatomy the following points should be kept in mind:

1. The topics should be of importance.
2. They should necessitate the study of structures which form part of many other functional groups, and which thus involve a knowledge of large portions of the body.
3. They should involve functions which have a demonstrable anatomical basis.

The respiratory system meets these requirements in a most satisfactory manner. It is of great importance; it demands the study of the entire thorax, a part of the head and neck, the spinal cord and spinal nerves, the vagus and trigeminal nerves, the sympathetic system, and the main sensory and motor tracts and centers of the brain; and finally, the correlation between structure and function can be shown in a most satisfactory manner. The alimentary system also is satisfactory in most respects. Although the correlation of structure and function is not easily shown in the portion below the diaphragm, in the portion above the diaphragm this can be shown very successfully.



The study of the anatomical structures of the alimentary tract involves many important relations in the head, neck, thorax and abdomen. The relation of the nervous system to swallowing and to mastication (taken in a very broad sense and including prehension and other acts preparatory to mastication proper) involves many reflexes in which practically the whole nervous system is utilized; of course the regulation of secretion and of blood supply should be included. Among other mechanisms which suggest themselves as suitable for study may be mentioned the maintenance of the erect position (including the part played by the nervous system in receiving, correlating and sending out impulses), the heart beat, the development and mechanism of speech together with the different forms of aphasia.

The course in correlational anatomy this year was limited to sixteen periods of three hours each; next year it will be extended. An outline of the course follows:

#### I. Respiration

- |   |          |    |  |
|---|----------|----|--|
| 1 | February | 19 | The thoracic wall and its movements                |
| 2 | February | 20 | Relations of the thoracic contents. Diaphragm      |
| 3 | February | 26 | Gross anatomy of nose, pharynx, larynx and trachea |
| 4 | February | 27 | Histology of respiratory system                    |
| 5 | March    | 5  | Mechanics of respiration                           |
| 6 | March    | 6  | Nerves and nervous centers                         |
| 7 | March    | 12 | Nervous reflexes                                   |
| 8 | March    | 13 | Summary  |

#### II. Mechanisms of the alimentary system

##### A. Mastication

- |    |       |    |  |
|----|-------|----|--|
| 9  | March | 19 | Gross and microscopic anatomy of the mouth |
| 10 | March | 20 | Nerves and nervous centers                 |
| 11 | March | 26 | Mechanism of mastication                   |

##### B. Deglutition

- |    |       |    |  |
|----|-------|----|--|
| 12 | March | 27 | Gross and microscopic anatomy of tongue, pharynx and esophagus |
| 13 | April | 2  | Mechanism of deglutition                                       |

##### C. Movements of stomach and intestines

- |    |       |    |  |
|----|-------|----|--|
| 14 | April | 3  | Gross and microscopic anatomy of stomach and intestines      |
| 15 | April | 16 | Movements and nervous mechanism of stomach and intestines    |
| 16 | April | 17 | Lecture  |
|    |       |    | (a) Sympathetic system                                       |
|    |       |    | (b) Innervation of viscera and of overlying muscles and skin |
|    |       |    | (c) Theory of emotions                                       |

In order to make such a course a success the instructor should carefully avoid each of two extremes. In the first place, the course may degenerate into a feeble attempt at a review in physiology in which the student, neglecting to study in actual preparations the anatomical structures upon which the various functions depend, spends his time over a book or in theorizing. In the second place, the student may become lost in a maze of anatomical details, losing sight of really vital anatomical facts and failing to bring these into relation with the activities which depend upon them. The instructor should possess above all a thorough knowledge of the anatomy and physiology of the nervous system; in addition he should be familiar with the gross and microscopic anatomy of the entire body and with general physiology, while a knowledge of pathology, clinical neurology, psychology and psychiatry will be of much value. With such a background of knowledge he may be expected to guide the student in forming a real conception of the various mechanisms of the human body.

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ANATOMY and THE ANATOMICAL RECORD.



## A USEFUL MODIFICATION OF MANN'S METHYL BLUE-EOSIN STAIN<sup>1</sup>

FRANKLIN P. REAGAN

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Mann's methyl-blue-eosin stain has recently been found to be very useful in the differentiation of embryonic tissues, especially in the study of the vascular system. Skillful manipulation of this stain gives a brilliant red to developing blood-cells, while other tissues are stained deeply blue. The stain is especially favorable for the study of haemapoiesis.

There are, however, certain defects in the original method. Constancy in the amount of differentiation in caustic alcohol is difficult to obtain; even different parts of the same embryo should be left in this reagent different lengths of time if a proper balance of red and blue be maintained throughout a given series. In the caudal region particularly it may be found that sections must remain for too short a time in caustic alcohol if enough blue be left in the tissue to be of greatest differential value. The nonvascular tissue is likely to possess a reddish color which cannot be removed by any reasonable amount of washing in distilled or acidulated water. With eosin present in all the tissues it is difficult to judge the amount of methyl blue which is being removed by caustic alcohol. These objectionable features may be largely eliminated by a few simple alterations of method. While these modifications have not received exhaustive trial, and while the stains so obtained have not yet been thoroughly tested for permanency, it is nevertheless true that they have afforded an astonishing degree and brilliancy of differentiation.

Sections are cleared in xylol, transferred through the alcohols to water, and then stained from forty-eight to ninety-six hours in Mann's mixture of:

One per cent aqueous solution of methyl blue.....	35 parts
One per cent aqueous solution of eosin <sup>2</sup> .....	45 parts
Distilled water.....	100 parts

Sections are rinsed in distilled water, then thoroughly dehydrated by direct transfer to absolute alcohol, after which they are differentiated in caustic alcohol made as follows: To each 30 cc. of absolute alcohol

<sup>1</sup>Mann, G. *Physiological histology*, 1902, p. 216.

<sup>2</sup>Grubler's aqueous eosin labeled 'W. GELBL' was found to be quite satisfactory.

are added five drops of a 1 per cent solution of caustic potash in absolute alcohol. Sections are removed from this solution when they have acquired a reddish-purple color. Following this they are rinsed in absolute alcohol, and then washed in distilled water, which, according to the original method should be allowed to remove the eosin from all the tissues except the blood-cells. In my own experience I have found that the eosin is sufficiently removed within five minutes or less; then they are transferred to the following mixture until over-stained:

One per cent aqueous solution of methyl blue.....	40 drops
Glacial acetic acid.....	30 drops
Distilled water.....	200 cc.

Sections are then washed in distilled water to remove acid, transferred to absolute alcohol, dehydrated thoroughly, de-stained in caustic-alcohol until the desired amount of blue is left in the tissue, rinsed well in fresh absolute alcohol, cleared in xylol, and mounted.

This gives the mesenchyme a clear blue, and the blood-cells varying degrees of bright red, depending on their developmental stages. Ganglionic and glandular structures tend to retain the purple obtained by the original method. Nerve-fibers and cartilage may assume a pea-green color. Endothelium of vascular plexuses may be made to take on a denser blue than the surrounding mesenchyme, rendering a plainness rivaling that obtained by injection. Also it might be mentioned that in the original method, an adequate amount of differentiation in basic alcohol tends to remove all blue from blood cells and their nuclei, so that a given field might exhibit cells all of which would apparently be eosinophilous. In the suggested modification such blue is restored to the basophile cells and nuclei.

Further modification of the amount of methyl blue added to the acidulated water or of the acidulation itself may effect further improvement, yet the present modification leaves little to be desired.

# THE MORPHOLOGY AND HISTOLOGY OF A CERTAIN STRUCTURE CONNECTED WITH THE PARS INTERMEDIA OF THE PITUITARY BODY OF THE OX<sup>1</sup>

ROSALIND WULZEN

*From the Hearst Anatomical Laboratory of the University of California*

SEVENTEEN FIGURES

Certain physiological experiments are now being conducted in the Rudolph Spreckels Physiological Laboratory of this University which necessitate the separation of a great number of ox pituitaries into their two main divisions. As an interesting anatomical feature was in this way brought to our attention the material was used in addition for this anatomical study. This feature has not been mentioned by the following who have written more or less fully upon the pituitary body of the ox, Peremeschko ('67), Dostojewski ('86), Herring ('08), and Trautmann ('11).

The pituitary body of the ox, like that of other vertebrates, is composed of two distinct portions. One, the pars nervosa, is derived from the brain as an outgrowth of the hypothalamus. The other originates as a hollow buccal evagination which in time is completely separated from the digestive tract. That portion of this evagination which comes into contact with the pars nervosa is called the pars intermedia. It is a comparatively thin sheet of epithelium which spreads as a coating over much

<sup>1</sup> Material amounting to thousands of pituitary bodies was most kindly supplied by the Oakland Meat and Packing Company through the courtesy of the Superintendent. It was derived from cows, bulls and steers. As the cone structure was present indifferently in these three varieties, its appearance can have little to do with sex or castration.



## ABBREVIATIONS

*P.N.*, Pars nervosa  
*P.G.*, Pars glandularis  
*P.I.*, Pars intermedia  
*C.*, Cone

*Cl.*, Cleft (residual lumen)  
*I.S.*, Infundibular stalk  
*B.S.*, Blood sinuses

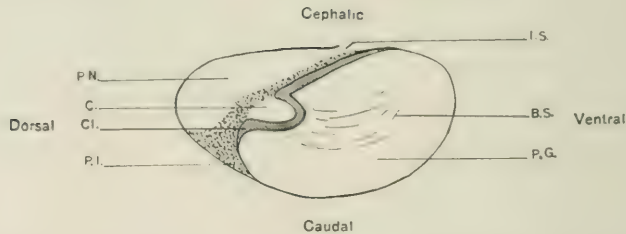


Fig. 1 Tracing of a mid sagittal section of ox pituitary, natural size. The pars glandularis is caudad and ventrad, the pars nervosa cephalad and dorsad. The pars intermedia lies between the two but is separated by the cleft from the pars glandularis. Note the cone upon the pars intermedia and the cavity of the pars glandularis into which it fits, also the blood sinuses coursing through the pars glandularis toward the cone.

of the pars nervosa. The cells in the remainder of the evagination proliferate greatly to form the pars glandularis, the bulkiest part of the pituitary body. Between the pars intermedia and the pars glandularis appears the remnant of the lumen in the original evagination. This is the residual lumen or cleft of the pituitary body. Figure 1 represents these parts as they appear in mid sagittal section. I have taken the side of the pituitary toward the brain to be 'cephalic,' that opposite 'caudal,' the side toward the nose to be 'ventral,' that opposite, 'dorsal.'

The pituitary body lies in the sella turcica roofed with thick dura mater which is perforated ventrally by the opening which transmits the infundibulum. Just underneath this covering is found the pars nervosa, a flask-shaped structure with a long narrow neck, the infundibulum, passing to the brain through the aperture in the dura. The pars glandularis surrounds the pars nervosa throughout its length and enfolds it on either side to such an extent that its only free portions are its dorsal extremity and a strip of its cephalic surface in contact with the dura. The cleft of the pituitary body is easily opened for examination. It

is found to have the same shape as the pars nervosa and thus the broadest portion is close to the dorsal extremity. In the greater number of cases the two walls of the cleft are closely approximated, but sometimes they are spread widely apart by the liquid or solid material which gathers in the cavity. Occasionally the main divisions of the pituitary are so extensively attached to one another that the cleft is obliterated except in its ventral extremity around the neck of the pars nervosa.

In the cleft there is almost invariably found a mass of tissue attached to the pars intermedia but very different from it. This structure is usually symmetrically placed in the mid sagittal plane one-third or less of the way from the dorsal to the ventral end of the cleft. Its general shape is that of a cone one side of which may be longer than the other. The cones differ in proportion; some are broad and low, even flat, others are tall and narrow at the base. Rarely there are two cones. The following are the dimensions of a few:

Length <i>mm.</i>	Breadth <i>mm.</i>	Height <i>mm.</i>
2	2	5
7	5	4
4	3	2
2	3	4
3	3	2
3	2	2

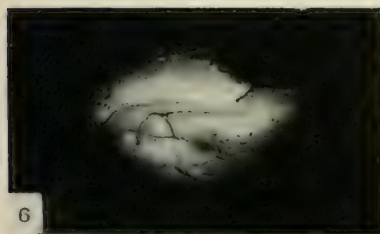
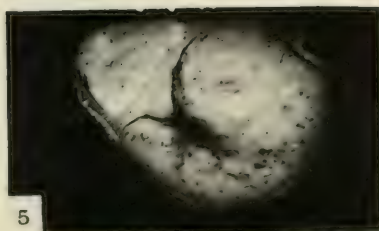
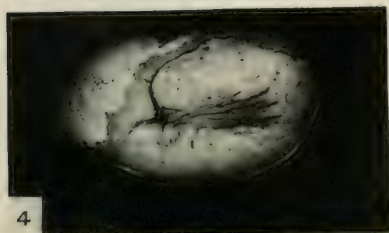
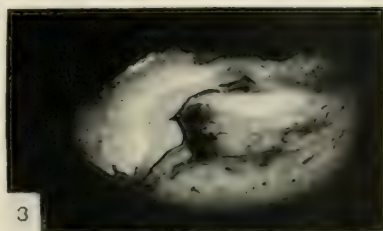
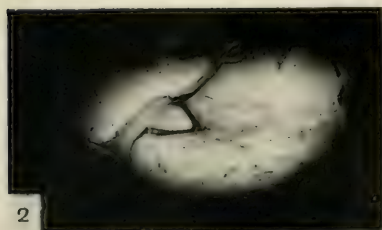
They vary from such sizes as the above to specks so small as to be just visible. Rarely they are not to be seen. Thus out of 760 tabulated cases 38 showed no such structure. Probably almost all of the 38 would have revealed it had a microscope been used. The writer has examined at least five thousand of these pituitaries and has come to the conclusion that the cone structure in some form is practically always present. Sometimes the cone has the shape of a bulb which is connected by a slender stalk with the pars intermedia, the whole structure being 8 or 9 mm. in length. In these cases the bulb may be so deeply imbedded in the pars glandularis as to reach almost to its opposite extremity.

Figures 2 to 7 show typical forms of the cone. The sections are approximately mid sagittal. By comparison with figure 1 their various parts may be easily identified. The bulkier and lower portion of each specimen is *pars glandularis*. The *pars nervosa* constitutes the larger part of the remainder. Between the two is the *pars intermedia* which is joined irregularly with the *pars nervosa* but is separated from the *pars glandularis* by the well developed cleft. Within the cleft is more or less colloid. Each specimen possesses a well marked cone which occupies a corresponding depression in the *pars glandularis* but is in no way attached to it. In figures 2 to 5 the cone is firmly attached to the *pars intermedia*. In figures 6 and 7 a line of division runs all around the cone separating it from *pars intermedia* as well as from *pars glandularis*. Probably in these the cone is attached to the *pars intermedia* by a slight strand of tissue which would be shown in a different section. Many cones partly separated in this way from *pars intermedia* have been found.

Occasionally the cone is shifted in position to the dorsal end of the cleft. It thus comes into contact with the *pars glandularis* and may be firmly attached to it. Figures 8 to 13 are examples of this arrangement. The sections are similar to the preceding ones, the difference being that here the cone is firmly attached to *pars glandularis*. Figures 8 and 9 show a transition between the forms preceding and those following. The larger part of each cone is separated from *pars glandularis* by the cleft but there is nevertheless an area of firm attachment to the tissue of the *pars glandularis*.

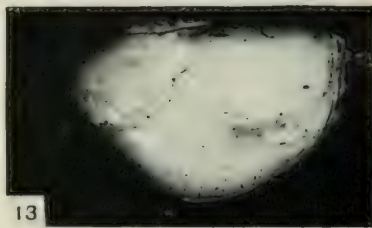
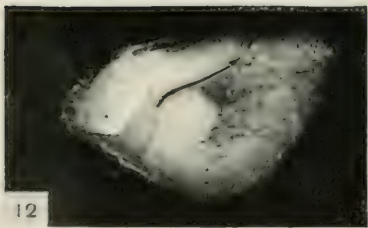
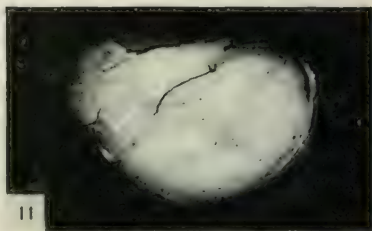
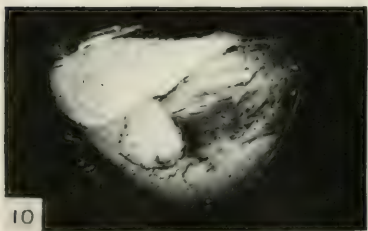
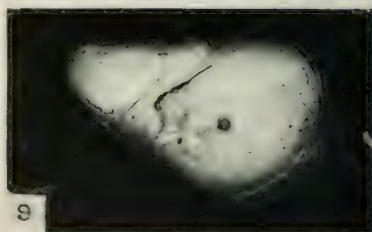
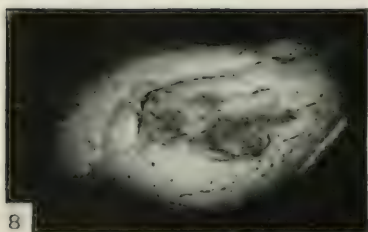
In order that the remaining specimens may be understood, it is necessary to notice the arrangement of the blood sinuses of the *pars glandularis*. When a sagittal section of the *pars glandularis* is made it is seen to have a dark core arching toward the cleft. This is composed of large blood sinuses. Though present elsewhere in the glandular substance they are most prominent here. They often stretch through the gland as an almost flat sheet in the mid sagittal plane and go with surprising directness to the cleft where they come to the surface in the mesial line and distribute themselves with the greatest liberality over the





Figs. 2 to 7 Approximately mid sagittal sections of ox pituitaries showing typical forms of the cone structure. Specimens were hardened in Zenker, stained lightly with alum-cochineal, and sectioned by hand. See description in text. Photograph  $\times 2$ . These and the following photographs were made by L. R. Newhart to whom the writer wishes to extend thanks.

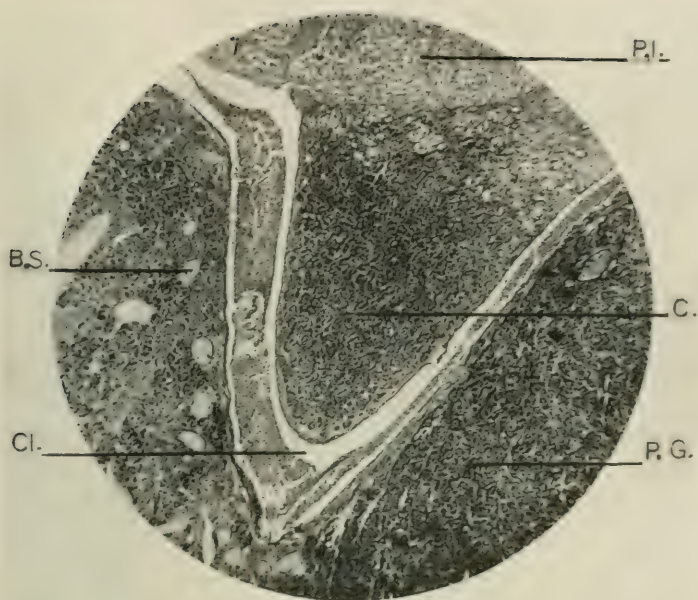
surface of the depression into which the cone fits. In every specimen examined the cone projects into this most vascular portion of the pars glandularis. The blood vessels are often so close to the surface in this region that they bleed with a touch.



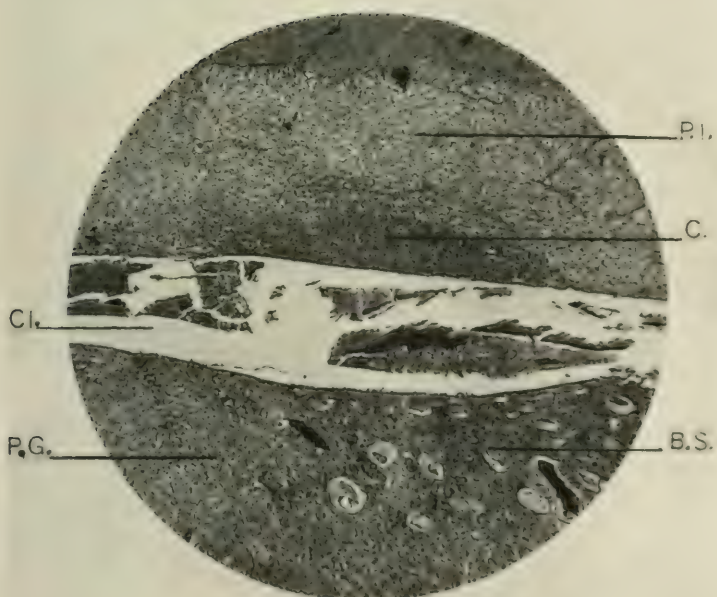
Figs. 8 to 13 Specimens prepared similarly to those in figures 2 to 7. These forms are somewhat exceptional. See description in text. Photograph  $\times 2$ .

Fig. 14 Mid sagittal section of ox pituitary in the region of the cone. Note the abrupt transition between pars intermedia and cone, cellular debris in the cleft, and numerous blood sinuses in pars glandularis. The band crossing cone and pars glandularis is an artifact. Section stained with Mallory's connective tissue stain. Photograph  $\times 25$ .

Fig. 15 Description the same as for figure 14. Here the cone tissue does not project into the cleft but it is seen to be as distinct from the pars intermedia as in the former case. Photograph  $\times 25$ .



14



15



In several of the specimens this core of blood vessels is distinctly seen as it travels to the exact location of the cone. In figures 10 and 11 the cleft appears only around the neck of the pars nervosa. The impression would be that there is no cone present were it not for the arrangement of the blood vessels. This is quite clear in both but is photographed most plainly in figure 10. Instead of arching to meet the pars intermedia at the apparent junction between pars intermedia and pars glandularis the blood sinuses come to an abrupt stop. In this way they mark out a large cone which is as well shaped as if it were separated completely from the pars glandularis by the cleft, no trace of which is here visible. In figure 11 the cone is marked out just as definitely. This also occurs in figures 12 and 13 but the outline of the cone is not so perfect, probably owing to incorrect section.

In color and consistency the cone is different from the pars intermedia. This difference which has been found to hold throughout the macroscopical examination is shown somewhat in the figures. The cone is composed of compact, creamy white tissue suggestive of the pars glandularis, whereas the pars intermedia is soft and more or less brown. The cone is often whiter and firmer than the pars glandularis itself.

#### HISTOLOGY

Pituitary bodies were fixed in Zenker's, Bensley's or Orth's fluids, were sectioned sagittally and stained with hematoxylin and congo red, eosin and methylene blue, or Mallory's connective tissue stain. Ten specimens were examined. Of these eight showed the cone structure undoubtedly present, and in one it was probably lost through poor technique. Figure 14 shows the cone projecting from the pars intermedia into the hollow of the pars glandularis. The cleft separates the two. That the tissue of the cone differs markedly from pars intermedia can be seen at a glance, the transition between the two being well marked. Figure 15 is a similar section of another specimen. The cleft runs through the center separating pars intermedia above from pars glandularis below. Here the cone tissue is raised only

slightly from the surface. It is, however, a fairly definitely circumscribed area which is strikingly different from pars intermedia. Even when the cone was so small as to be composed of a few cells only, its appearance was unmistakable. Greater magnification reveals the reason for the difference. Cone tissue contains as its most striking characteristic, deeply staining acidophile cells with coarsely granular protoplasm which are apparently identical with those of the pars glandularis. As far as I have been able to determine, acidophile cells have never before been noticed so closely associated with the pars intermedia. Indeed, Herring ('08) says "The intermediate portion, although derived from the same source as the main anterior lobe (pars glandularis), differs from it in adult mammals in that it contains no eosinophile cells." Tilney ('11) joins him in the remark, "The juxta-neural epithelial portion (pars intermedia) is invariably basophilic." These statements have great weight in that both investigators have made careful examinations of the pituitary bodies in many different animals. The remaining cell elements of the cone are similar to those of the pars glandularis. But certain differences exist which, though perhaps of slight importance, make it possible to distinguish between pars glandularis and cone tissue. The connective tissue septa are finer and the interstices are ordinarily smaller and contain fewer cells in the cone than in the pars glandularis. On this account the grouping of the cone cells into acini may appear to be lacking, while the acini of the pars glandularis are apparent at a glance (figs. 16 and 17).

One of the specimens examined was found to resemble the condition shown in figures 10 and 11. The cone was firmly joined to pars glandularis as well as to pars intermedia. Microscopically it showed itself to be as typical a cone as any of the others. A definite connective tissue septum outlined the surface of the cone, adjacent to the pars glandularis. Its tissue differed in the same manner as the other cones from the surrounding tissue of the pars glandularis.

It is noteworthy that in the only specimen in which the cone was actually lacking the cleft was very small, appearing only

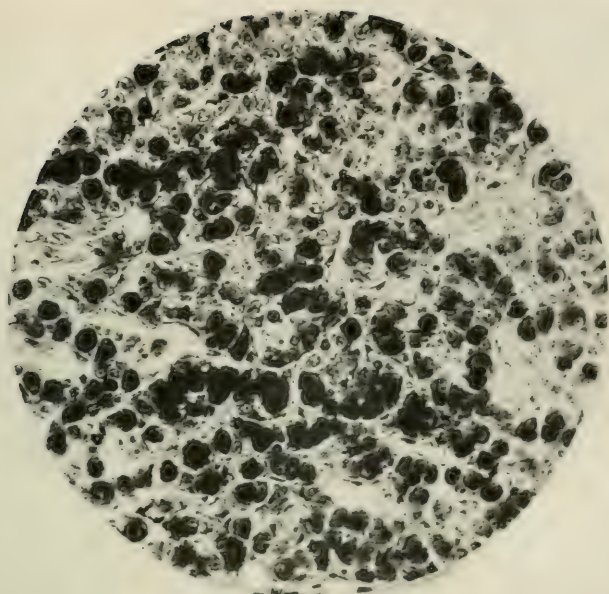
around the neck of the pars nervosa. The pars intermedia over-lapped the cleft at its dorsal end and spread irregularly some distance into the pars glandularis. Also large portions of the pars glandularis had the characteristic appearance of cone tissue, that is, the acini had few cells and were separated by very fine strands of connective tissue. In no other specimen did the pars glandularis have this appearance.

Microscopically the vascularity of the cone appears to be slight but in spite of this when the cleft of a fresh specimen is opened the cone is often flushed with blood. The blood vessels appear to be superficial and are spread mainly about the base of the cone.

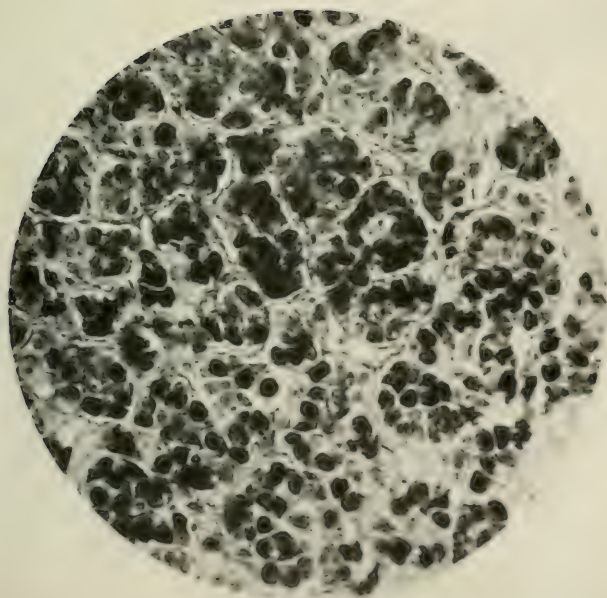
This preliminary work leaves many problems unsolved. What are the phylogenetic relationships of this very constant structure? Can any light be thrown upon it by the study of its development? Has it any physiological significance? If this study suggests anything it suggests a closer relationship between pars glandularis and pars intermedia than has hitherto been suspected. The distribution of the blood sinuses of the pars glandularis about the cone is often so striking that it is impossible to avoid the impression that such an appearance cannot be an anatomical chance. Thus when the cone penetrates deeply into the pars glandularis, the walls of the cavity into which it is so closely fitted are solidly packed with blood sinuses. This would appear to be an excellent arrangement for the absorption into the blood stream of any secretions from the cone or pars intermedia. Certain color changes should also be mentioned in this connection. The pars intermedia is ordinarily distinctly tinged. It assumes all colors between dull white and bright orange or yellow or brown. The pars nervosa never shares its color. It is a uniform dull white. On the other hand the pars glandularis may have its characteristic creamy or

Figs. 16 and 17 Detail from the specimen shown in figure 14. Figure 16 shows typical tissue of the cone, figure 17 typical tissue of pars glandularis. Note the prominence of the deeply staining acidophile cells in both, but observe that in the pars glandularis the acini are more prominent and the connective tissue septa are broader. Photograph  $\times 275$ .





16



17

grayish white appearance when the pars intermedia is brightly colored or it may share the coloration of the pars intermedia. Thus it too is sometimes a deep orange. It, however, has never been observed to be highly colored unless the pars intermedia as well is brightly colored. This also suggests a common activity of pars intermedia and pars glandularis or some interrelation between them. Work is being carried out along the lines suggested.

Acknowledgment is due to Dr. P. E. Smith for his kindly help.

#### CONCLUSIONS

1. A structure of more or less definite cone shape appears constantly upon the pars intermedia of the ox pituitary.
2. Its cellular elements resemble those of the pars glandularis. Numerous acidophile cells are its most striking feature.
3. It differs from pars glandularis through having in a general way finer connective tissue septa and smaller acini.

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## OSSICULUM LUS

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Some reference to this mysterious bone is found in all anatomies of ancient times and in those of the Middle Ages. The name itself is derived from the Hebrew 'luz' (almond), Arabian, 'lauz.' Its history may be of some interest.

In the Old Testament (1) we find the following passage: "Custodit Dominus omnia ossa justorum—unum ex illis non confrigitur." In the English version we read: "Not one of them is broken," but there is no emphasis on the 'one,' nor need it mean, as the Latin renders it, "there is one which shall not be broken," for had the Hebrew writer meant simply to say, "none of his bones shall be broken," he would have stated it in the same manner. Reference to both Isaiah, xxxiv. 16, and Psalm cvi, 11, shows the use of the same idiom. In neither of these passages is it to be understood that a particular one (bird or enemy) is missing.

Further, we may say that not merely may Psalm xxxiv, 20, be translated as in the English version, but that it must be so translated. The two clauses of the verse, as so often in Hebrew poetry, contain parallel ideas, simply repeating the same thought in different words, thus:

He keepeth all his bones,  
Not one of them is broken.

In this psalm there is no thought of any life after death. It is in this life that God preserves the righteous. Nevertheless, the possible alternative translation of the Hebrew affords a point for argument, and it is probable that the rabbinic mind, apt to controversy, has raised the question whether or no the translation should be 'one of them' with the emphasis on the 'one,' thereby stimulating the search for the 'unum os' now well-known as the 'os luz.'<sup>1</sup>

The learned Rabbi Usaia (2), who lived in the third century A.D., defined this bone as "in fine octodecim vertebrarum." Not only Usaia, but other writers (especially the anatomists of the School of Salerno) have given the number of vertebrae as eighteen (3): "Sunt autem octodecim spondilia, in colle sex, in dorso duodecim." According to Haller (4) the lumbar vertebrae were not considered vertebrae proper, because they do not enclose any part of the spinal cord, but only the cauda equina of the talmudists. Even at that, as Hyrtl (5) remarks, the number of vertebrae would be 19 and not 18.

<sup>1</sup> For help in the translation and interpretation of the literal Hebrew, I would acknowledge my indebtedness to Dr. W. F. Moulton and Rev. James Todd.



Bauhinus (6) quotes a passage from a commentary on Ecclesiastes: "We read in the commentaries of the Rabbis, that the Emperor Hadrian asked R. Jehosua, the son of Chanina, out of what God would resurrect man in the life to come, to which he replied, 'Out of the Os Lus of the vertebral column;'" ("Apud Rabbinos legitur, 'Adrianum Caesarem interrogasse R. Jehosuam, filium Chaninae, unde Deus hominum in futuro saeculo erecturus sit, respondisse, ex osse Lus spinæ dorsi'").

The 'os lus' was considered incorruptible by the talmudists, as may be seen from a quotation by Hyrtl (5): "A bone which cannot be harmed by water, by fire or by any other element; which cannot be broken or crushed by any natural force; which God will sprinkle with heavenly dew in the last judgment; then all the other members will be gathered unto it and form again one body, which, through the inspiration of the Divine Spirit, shall take to itself new life"; ("Os quod neque aqua, neque igne, neque alio elemento corrumpi, nec ulla vi externa frangi aut conteri possit, quod os Deus in extremo judicio coelesto rore irrigabit, et tunc reliqua membra ipsi rursus aggregabuntur, et in corpus coalescent, quod spiritu Dei afflatus, vivum resurget").

This bone is further described in the book *Adam Siehti* (i.e., *homo intellectualis*) Article XIII: de resurrectione mortuorum, quoted by Bauhinus (6): "Never can this bone be burned or destroyed, for its origin is in the Heavens; from it, moistened with dew will God restore the dead to life as from leaven which is hid in a measure of meal"; ("Hoc os non comburi, nec corrumpi in perpetuum potest, quia radix ejus est ex substantia coelesti, et humectatur rore, quo Deus resuscitaturus sit mortuos, tanquam fermento, quod est in massa farinae").

Baal Aruch, quoted by Rolfink (7) says in regard to this bone: "The whole body of man decays, save that one bone whose form is like unto an almond;" (*Totum hominis corpus putrescit, excepto illo osse, estque simile amygdalæ.*)

Thus the 'ossiculum lus' received also the name 'os' or 'semen resurrectionis.' Its incorruptibility was not only believed to exist, but to have been proved: The Emperor Hadrian, after Jehosua had told him that God would resurrect the dead out of the 'os lus,' asked for the proof of this assertion; and according to Bauhinus (l.c.): "Into the Emperor's presence Jehosua bade them bring this bone, which thrown into water was not softened, laid upon the fire was not burned, brayed in the mortar was not crushed, set upon the anvil and beaten with the hammer, suffered no harm though the anvil was burst asunder"; (*R. Jehosua os illud in conspectum ejus afferri curasse, quod aquae impositum, non fuit emollitum,—igni, non fuit adustum,—molae, non fuit attritum,—incudi,—et percussum malleo, rupta fuit incus, os autem nullum defectum sensit*").

Cornelius Agrippa (8) adds: "This little bone, called *luz* by the Hebrews, in size but that of a pea, touched by no decay, nor conquered by the fire, will remain unblemished forever; from it, as a plant from the seed, our body shall arise in the resurrection of the Dead," which

sentence he ends with these remarkable words: "Et hae virtutes non declarantur ratione, sed experientia;" ("Os minimum, quod Hebraei Luz appellant, magnitudine ciceris mundati, nulli corruptioni obnoxium, nec igne quidem vincitur, sed semper conversatur illaesum, ex quo, velut planta ex semine, in resurrectione mortuorum, corpus nostrum repullascet").

An editorial in the *Lancet* (9) refers to this bone as the twelfth dorsal vertebra, "the turning point and centre of the spine." Although no reference is made to the source of this information, I think the quotation is taken from Galen, who in all probability dissected only animals, and it would thus refer to the 'anti-clinal' vertebra.

Since this remarkable bone could not be found "in fine octodecim vertebrarum," search was begun elsewhere. The skull as an important part of the body attracted attention first, and the supernumerary bone, frequently found at the junction of the sagittal and lambdoidal sutures (perhaps the os interparietale or os Incae?) was thought by some to be the 'os lus.' Many virtues were attributed to this bone, and its remedial powers were supposed to be great, especially when it came from the skull of an executed criminal. According to Hyrtl (14), the pulverized bone was first used as a remedy for epilepsy by the Swiss physician Höchner, who latinized his name to Paracelsus, and hence the term, *ossiculum antiepilepticum Paracelsi*.

A similar term was used in reference to the os epiptericum (Virchow) os epilepticum, which, according to Lombroso, is always to be found in delinquent and demented people (23).

But not all skulls have these epactal bones, and thus other writers looked for it at the base of the skull, as Hieronymus Magius (10) tells us, without stating, however, which bone it is. According to Bauhinus (l.c.), the seventh cervical vertebra, the vertebra prominens; according to Dassovius (11), the os coccygis was taken for the 'ossiculum lus.' The explanation of Dassovius is very probably based upon the Arabian name for coccyx, 'al ajab' (al ajas), "which Mohammed stated to be incorruptible and to serve as the basis for the future edifice" (9).

The same author (9) informs us that the os sacrum was thought to be the 'os lus' "on account of its old name *ἱερὸν ὀστέον* but here the author makes the same mistake which many others made before him. Isidorus (12) gives the following explanation of the os sacrum: "Ima spinæ pars, quam Graeci *ἱερὸν ὀστέον* vocant, quia primum in infanti nascitur, ideoque et hostia a gentilibus diis suis dabatur."

The word 'sacer' is explained by Festus (13) in the following manner: "Gallius Aelius declares sacred (sacer) that which in any manner is dedicated to the state, whether house, altar, eagle, riches or anything dedicated and consecrated to the gods." ("Gallius Aelius ait, sacrum esse quoquunque modo atque instituto civitatis consecratum sit, sive aedis, sive ara, sive signum, sive pecunia, sive quid aliud diis dedicatum atque consecratum sit").

Marcus Aurelius: "Anything set apart for the gods is termed sacred (sacer)," and "Sacred (sacer) are those things which have been conse-

erated to the gods above;" ("Quidquid destinatum est diis 'sacrum' vocatur;" "Sacrae (res) sunt quae diis superis consecratae sunt." Institutiones juris civilis).

All these definitions of 'sacer' do not explain the 'sacrum' in 'os sacrum'; there are, however, other possible explanations: The first is contained in the statement made by Hyrtl (14), that the 'os sacrum' is simply an erroneous translation of the Greek *ἱερόν ὀστέον*.

1. The Greek term for 'os sacrum' was *σπόνδυλος μέγας* or *ἱερός (ὀστέον μέγα* or *ἱερόν)*, where *ἱερός* has the meaning of 'magnus;' thus Homer uses "Ἴλιος ἱρή and *ἱερός πόντος* (for "Ἴλιος μεγάλη and *μέγας πόντος*). Spigelius (15) says: "Graecis omnia magna 'sacra' vocabantur;" and Caelius Aurelianus (16): "Majora omnia vulgus 'sacra' vocat."

2. There is, however, in Latin itself an explanation of 'sacer' which seems to me preferable and more plausible, namely, its meaning 'detestable,' as we find it in the *Leges XII tabularum*: "That man is anathema (sacer) whom the people have found guilty of a crime; and "an advocate shall be detestable (sacer) who defrauds his clients"; ("Homo 'sacer' est quem populus iudicavit ob maleficium;" and "patronus qui clienti fraudem fecerit 'sacer' esto" (ibid).

According to this definition of 'sacer,' 'sacrum' would be the equivalent of 'detestandum,' and the bone received its name 'sacrum' (i.e., detestandum) from its being near the rectum (obscena).

3. In addition to this Garrison (17) quotes Ramsbotham (18), who suggests that *ἱερόν* in connection with the sacrum is not the Greek *ἱερόν*, but a corrupted form of the Hebrew 'heron,' signifying conception, parturition, whence also Hera, the goddess of childbirth.

Garrison (l. c.) also produces some evidence that the external sesamoid bone of the great toe was thought by certain authors to be the 'ossiculum lus.'

Lastly, the inner, larger sesamoid bone of the *Articulatio metatarso-phalangea hallucis* was selected as the *os resurrectionis* on account of its real hardness and its form (seed of the sesamum); it is mentioned by Vesalius (17), Riolanus (18) and Bartolinus (19) under the name 'albadaram,' and as such it played a great rôle "apud magiae et occultae philosophiae cultores."

The *os sacrum*, however, as the mysterious *ossiculum lus*, has found its place in history in the 'rump parliament,' as may be seen in the following quotation from Butler (22):

The learned Rabbis of the Jews  
Write there is a bone they call Luz  
I' the rump of man, of such a virtue,  
No force of nature can do hurt to:  
And therefore at the last great day  
All th' other members shall, they say,  
Spring out of this, as from a seed  
All sort of vegetals proceed:

From whence the learned sons of art  
*Os sacrum* justly style that part.  
Then what can better represent  
Than this Rump Bone, the Parliament  
That after several rude ejections,  
And as prodigious resurrections,  
With new reversions of nine lives  
Starts up and like a cat survives?



But the name has disappeared from anatomical text-books, and the word remains in our dictionaries only as a reminder of the anatomy of times past.

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- (4) HALLER: Bibliotheca anatomica, T. 1, 2, p. 126.
- (5) HYRTL: Das Arabische und Hebräische in der Anatomie, p. 166.
- (6) BAUHINUS: Theatrum anatomicum, Lib. 1, cap. 48.
- (7) ROLFINK: Dissertationes anatomicae, Lib. 2, cap. 54: De ossibus sesamoideis.
- (8) CORNELIUS AGRIPPA: De occulta philosophia, Lib. 1, cap. 20.
- (9) THE 'OS LUS'. Lancet, vol. 2, 1910, p. 1029.
- (10) HIERONYMUS MAGNUS: De mundi exustione et die judicii, Lib. 5, cap. 1.
- (11) DASSOVIVS: Tractatus de resurrectione mortuorum, cap. 3, p. 23.
- (12) ISIDORIUS: Etymologicorum, Lib. 2, cap. 1.
- (13) FESTUS: De verborum significatione (letters M-V) ed. Müller.
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- (15) SPIGELIUS: De corporis humani fabrica, Lib. 10.
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- (17) GARRISON: The bone called 'luz,' New York Med. Jour., vol. 92, p. 147.
- (18) RAMSBOTHAM: Obstetric medicine and surgery, p. 698.
- (19) VESALIUS: De corporis humani fabrica, Lib. 1, cap. 28.
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- (21) BARTHOLINUS: Institutiones anatomicae, Lib. 4, cap. 22.
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- (23) LE DOUBLE: Traité des variations des os du crâne, p. 306.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

PSYCHOLOGY IN DAILY LIFE (Conduct of the Mind Series, edited by Joseph Jastrow), by Carl Emil Seashore, Professor of Psychology and Dean of the Graduate College in the State University of Iowa. 226 pages, New York, 1914, D. Appleton and Company, \$1.50 net.

Publisher's Announcement. This volume well represents the general purpose of the Conduct of Mind series which is, to present for the intelligent reader the several aspects of mental affairs which are involved in the regulation of practical interests. The volume comprises a selection of illustrative material with their interpretation, and may well serve as an introduction to the study of psychology. It proceeds by selecting a few general topics rich in application and about which a considerable range of mental principles may be grouped. The several chapters deal with topics such as Play, The Law in Illusion, Mental Measurement, Mental Health and Mental Efficiency. The illustrations are in each case given a sufficient setting so that they become typical of the problems of psychology and at once suggest how competently the issues of our daily life are conditioned by the psychological basis. The work is free from technical terms and presents a fresh and original arrangement of the material characteristic of modern interest in the laws of the mind.

## PERSISTENT ARTERIAE BRACHII SUPERFICIALIS. ANTIBRACHII SUPERFICIALIS ET MEDIANA

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### ONE FIGURE

An unusual artery found in the left arm of a man of thirty-seven years seems worthy of record.

The vessel emerges from the axillaris midway between the aa. subscapularis and thoracalis lateralis, on the median side. It runs in the deep fascia anterior and medial to the a. axillaris, the a. brachialis and the n. medianus, almost to the middle of the humerus, where it crosses the a. brachialis and the n. medianus, to enter the m. biceps brachii from beneath, through two large divisions.

It gives off two small cutaneous rami in the lower axillary and upper brachial regions. In size the artery is about two-thirds that of the normal subscapularis until it reaches the biceps muscle. At this point it gives rise to a small ramus almost at right angles to it. This courses lateral and anterior to the brachial artery in the deep fascia, becomes superficial at the elbow, and continues anterior to the ulna, to the palm. Here it enters into formation of the arcus volaris superficialis, together with the a. ulnaris. The arch has no connection with the a. radialis.

There is no ramus of the a. ulnaris or a. interossea which may be called an a. mediana. The a. mediana described in this paper has no relation to the n. medianus, which is placed deep in the forearm.

The embryological significance of the artery in question may be derived from Müller's<sup>1</sup> figure of the arteries in the arm of an

<sup>1</sup>Müller '03, Anat. Hefte, Bd. 22, Taf. 25-26, fig. 9.



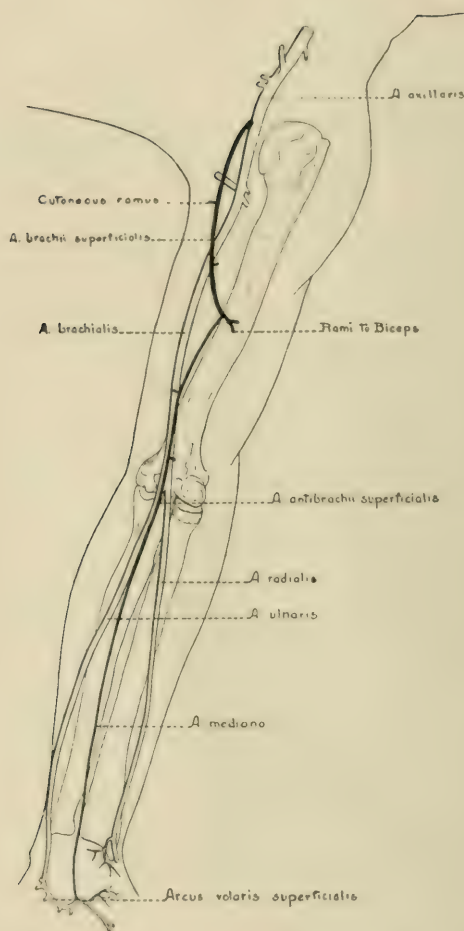


Fig. 1 Persistent arteriæ brachii superficialis, antibrachii superficialis et mediana. The rami of the aa. brachialis, radialis and ulnaris are not shown in the figure, as they are normal except for the two discrepancies noted.

11.7 mm. human embryo ('03). From this figure it would seem that we have a persistent a. brachialis superficialis, giving rise to an a. antibrachii superficialis, which becomes an a. mediana, but all anastomoses with the a. brachialis have been lost. As stated by some texts of anatomy, this condition is one that is quite rarely found.

# THE MICROSCOPIC STRUCTURE OF MAMMALIAN CARDIAC MUSCLE WITH SPECIAL REFERENCE TO SO-CALLED MUSCLE CELLS

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EIGHT FIGURES

In recent histologic descriptions of the mammalian heart the muscle is more commonly regarded as a syncytium. The unique characteristic of heart muscle is the presence of intercalated discs. The earlier interpretation that these discs mark cell boundaries has been most recently championed by Zimmermann (1). That cardiac muscle, however, can not be regarded as composed of cells separated from one another by 'intercellular' intercalated discs I have attempted to prove in a recent series of papers (2, 3, 4, 5). The salient observation among the counter-vailing facts recorded concerns the occasional supernuclear position of these 'discs.'

In 1888 Apathy (cited from Lewis, 6) advanced the interpretation that striped muscle, including heart muscle, was structurally comparable with the connective tissues, consisting of cells and extracellular bundles of myofibrillae. Recently Baldwin (7, 8) has attempted to establish this hypothesis upon a basis of cytologic observations, and concludes that voluntary striped muscle generally, and cardiac muscle of the adult white mouse, consists of distinct muscle cells, and extracellular columns of muscle fibrils and sarcoplasm enveloped by sarcolemma. The 'cells' are described as lying outside the sarcolemma.

If Baldwin's conception of cardiac muscle is correct, then his observations contribute one of the strongest objections to any interpretation that considers the intercalated discs as intercellular structures marking cell boundaries.

I shall concern myself here chiefly with Baldwin's conception of striped muscle structure as it pertains to cardiac muscle, my special interest being enlisted by reason of its bearing on the nature of the intercalated discs.

Baldwin used only sectioned material. The essential point in his proof that there are 'muscle cells' pertains to the presence of a delicate 'cell membrane,' separating a nucleus with an envelope of cytoplasm from the myofibrils imbedded in a distinct sarcoplasm. Such conclusion presupposes very delicate observations. One must guard against fixation artefacts and misleading appearances due to obliquity of section. Obviously from this standpoint macerated tissue is preferable to sectioned tissue. In macerated preparations one can examine considerable lengths of single muscle trabeculae, and by careful focussing thus view exact median longitudinal sections (optical) of 'fibers,' obviating all errors due to obliquity of section. Also, in properly preserved specimens shrinkage is prevented; and even a fair degree of differential staining can be obtained.

For the purpose of my study I employed principally dissociated tissue of fresh cat's heart; also macerated tissue of previously fixed (in Carnoy's fluid) heart of white mouse. I had on hand also abundant sectioned and stained material (treated according to Zimmermann's technic) of various mammals, and of heart of adult white mouse (Carnoy's fixation; iron-hematoxylin-eosin stain) for additional study.

The cat tissue was macerated in a saturated solution of potassium chlorate in nitric acid and preserved in a mixture of equal parts of water, 95 per cent alcohol and glycerin. Many stains, both cytoplasmic and nuclear, and various combinations of stains were employed. The best results were obtained by use of borax carmine or eosin in various degrees of concentration.

That the method of treatment does not seriously injure the tissue for detailed observation is indicated by figure 1 which purports to be an accurate representation of actual appearances in the median longitudinal plane. The sarcolemma is well preserved as shown in the lower portion of the illustration where it is festooned between successive telophragmata (Krause's



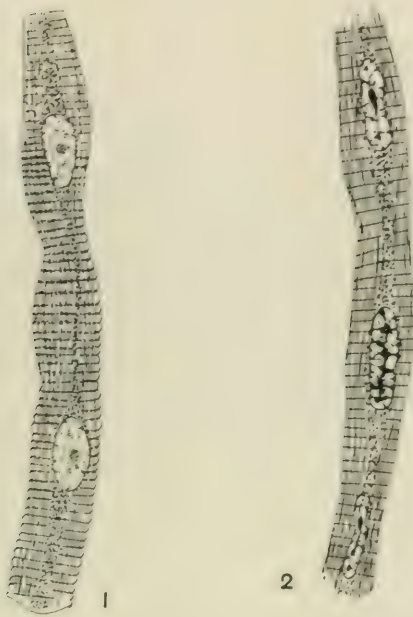


Fig. 1 Muscle fiber of cat's heart from macerated preparation, showing two nuclei connected by a continuous axial strand of coarsely granular sarcoplasm. The drawing represents appearances in the optical median longitudinal plane. There is no evidence of a cell membrane separating the central granular from the peripheral non- or finely-granular sarcoplasm.  $\times 1500$ ; reduced one-third in reproduction.

Fig. 2 Median longitudinal section of muscle fiber from ventricle of adult white mouse showing three nuclei imbedded in a continuous axial strand of coarsely granular sarcoplasm. The specimen was fixed in Carnoy's solution, and stained with iron-hematoxylin and eosin.  $\times 1500$ ; reduced one-third in reproduction. The destaining process is here carried too far to show the intercalated discs.<sup>1</sup>

<sup>1</sup> Fixation in Carnoy's strong solution (acetic alcohol with chloroform), followed by iron-hematoxylin staining, is a good technic for demonstrating intercalated discs. This method brings out sharply also the Q-substance and the Z-lines. The phase of contraction is thus clearly shown. For a study of the relation of the discs to 'contraction bands' it seems preferable to any of the special methods for intercalated discs.

membranes, of Z discs). In the upper portion of the figure the festooning does not appear. From the standpoint of the myofibrils the fiber differs in different regions. In the mid-portion, which is constricted (contracted), the fibrils are coarser and show a distinct alternation of light and dark bands (discs). In these same regions also the telophragmata are much coarser. The coarser telophragmata and the darker discs of the stouter fibers are identical, and represent 'contraction bands of Rollet.' The nuclei are clear with sharp contour, each surrounded by coarsely granular cytoplasm. The technic has clearly preserved such delicate structures as sarcolemma, differences between contracted and relaxed portions of the fiber, nuclear wall and reticulum, and cytoplasmic (sarcoplasmic) reticulum and granules.

But in face of these facts no definite indication of a cell membrane appears. The perinuclear sarcoplasm shades away into the peripheral fibrillar and finely granular sarcoplasm without sharp line of demarcation except such as is simulated at certain levels of focus by adjacent myofibrillae. Moreover, one perinuclear cone of sarcoplasm connects with adjacent cones through narrow strands of structurally similar sarcoplasm. In sections of such trabeculae a slight obliquity of cut would give an entirely false impression. This observation of a continuity of axial undifferentiated sarcoplasm, swelling at the levels of the nuclei, can be made only very rarely in sectioned tissue. However, figure 2 shows a similar condition in a section of ventricle of adult white mouse. In cross-sections of cardiac muscle of various mammals such a central core is almost invariably discernible, though frequently so frail as to escape notice unless specially looked for.

The undifferentiated sarcoplasm forms an axial granular core; the telophragmata extend through it, though frequently somewhat irregularly, as can be clearly observed in tissue deeply stained with iron-hematoxylin. There is no evidence warranting a distinction between the perinuclear plasma as cytoplasm, and the myofibrillar portion as sarcoplasm. No clear evidence appears of a cell membrane in macerated tissue; in fixed tissue an adjacent myofibril, not showing a clear alter-

nation of light and dark discs, or a condensation (fixation artefact) of peripheral protoplasm, may simulate a cell membrane. Such apparent 'cell membranes' can frequently be traced at some distance into an undoubted fibril. Nor is there any indication of the complicated investment of sarcolemma with respect to myofibrils as conceived by Baldwin. The main observations, however, arguing against Apathy's original conception are the continuity of the axial strand of granular undifferentiated sarcoplasm, and the continuity of the telophragmata throughout the extranuclear portion of the muscle.

But granted that fusiform heart muscle cells actually do exist as illustrated by Baldwin: such cells should then appear isolated in properly macerated material. On the contrary one finds only such short fragments as illustrated in figure 3. Fractures occur in the macerating fluid along the telophragmata, frequently at the levels of the intercalated discs. Such fragments suggest a close structural association between the granular perinuclear sarcoplasm and the non-granular sarcoplasm among the fibrils, most probably by virtue of the telophragmata as first described by MacCallum (9). When the maceration has progressed further only naked nuclei appear (fig. 4), with small adherent masses of sarcoplasm. Occasionally such a structure as illustrated in figure 5 appears. Here a peripheral coarse fiber-structure simulates a portion of a cell membrane. But usually its striped character reveals its myofibril nature.

That the technic does actually isolate cells when present is shown by abundant spindle shaped cells (fig. 6) from the endomysium. If the muscle nuclei and spindle shaped areas of enveloping sarcoplasm actually constituted spindle shaped cells surrounded by a membrane, the same technic which isolated such structures from the connective tissue of the same material would also be expected to isolate them from the muscle complex. The hypothetical spindle shaped cell of cardiac muscle is obviously structurally not closely similar to the fusiform cells of the endomysium, or of smooth muscle.

Smooth muscle from the intestine of the cat subjected to an identical technic yields the usual fusiform cell, enclosed in a





Fig. 3 Fragment of fiber from macerated cat's heart, drawn as if it were a transparent object. The nucleus is surrounded by granular sarcoplasm. The breaks follow telophragmata, without any relation to hypothetical cell membranes. The manner of fracture strongly indicates that the central granular and more peripheral non-granular sarcoplasm are continuous, probably by reason of the meso- and telophragmata, and that the former is not invested by a cell membrane.  $\times 1500$ .

Fig. 4 Naked nucleus from similar preparation, with adherent clumps of sarcoplasm.

Fig. 5 Nucleus with an adherent mass of granular sarcoplasm from the same preparation. The sarcoplasm is delimited at the right by a stout fibril which simulates a membrane, but a faint segmentation reveals its true myofibril nature.

Fig. 6 Isolated fusiform connective tissue cell of the endomysium from the same preparation.

Fig. 7 Isolated large fusiform smooth muscle cell from cat's intestine. The nucleus is surrounded by coarsely granular sarcoplasm as in cardiac muscle, which is continuous similarly with the more peripheral non-granular or finely granular sarcoplasm, though myofibrils may simulate, as in heart muscle, a cell membrane.  $\times 1500$ ; reduced one-half in reproduction.

Fig. 8 Oblique transverse section of smooth muscle cell from muscularis mucosae of esophagus of cat. The perinuclear sarcoplasm has contracted away from the nucleus leaving a clear space, peripherally limited by a sharp line, a fixation artefact, simulating a cell membrane. The light-blue-staining sarcoplasm however is peripheral to this 'membrane.' Zenker's fixation; hematoxylin-eosin stain.  $\times 1500$ .

distinct membrane. The centrally placed elongate nucleus is enveloped by undifferentiated granular sarcoplasm in a like manner, and of apparently identical structure, even to a delicate peripheral 'membrane,' as in cardiac muscle trabeculae. Further mechanical treatment (teasing) separates a similar essentially bare nucleus. Fixed smooth muscle stained with the hematoxylin-eosin combination shows the central perinuclear mass of sarcoplasm stained a faint blue, in contrast to the deep blue of the nucleus and the bright red of the cytoplasm. Certain cells show contraction artefacts. In these cases a space, empty, except for occasional very delicate strands, appears between nucleus and contracted cytoplasm. The inner surface of the latter exhibits a sharp contour, simulating a delicate membrane.

In cross-sections one finds appearances like figure 8 (oblique section of smooth muscle fiber of muscularis mucosae of esophagus of cat). Occasional strands spanning the space might be interpreted as spongioplasm; but the space is colorless, while the light-blue-staining sarcoplasm is without but closely applied (indicating contraction) to the peripheral border or 'membrane' of the space.

If cardiac muscle can be appropriately interpreted in terms of fusiform cells and extracellular masses of myofibrils and sarcoplasm, smooth muscle should be similarly interpreted, since apparently exactly the same cytologic conditions as regards nuclear relation to sarcoplasm prevails in both, irrespective of course of telophragmata. But the histogenesis of smooth muscle renders such interpretation very improbable. Moreover, maceration separates the genetic units, not secondary structures. Similarly in the case of cardiac muscle: genetically we start with a syncytium in which myofibrillae are deposited; maceration separates irregular fragments, and ultimately yields naked nuclei and masses of myofibrillae imbedded in sarcoplasm. Since the intercalated discs, locations where fragmentation frequently takes place, can not be regarded as cell boundaries, the cardiac muscle must be conceived to persist in its original syncytial condition.

The results of this study of cardiac muscle by the dissociation method, and comparative observations of similarly treated endomysium and smooth muscle tissue, yield no evidence in favor of the cellular conception of heart muscle suggested by Apathy and supported by Baldwin. On the contrary heart muscle appears to be a true syncytium, the anastomosing muscle trabeculae consisting of axial strands of undifferentiated coarsely granular sarcoplasm containing nuclei, and peripheral layers of apparently non-granular or finely granular sarcoplasm differentiated in that it contains myofibrillae marked by alternating dark and light discs and intercalated discs, the telophragmata being continuous throughout the extranuclear muscle complex.

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## THE THYROID GLAND OF THE OPOSSUM

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### THREE FIGURES

In the thyroid glands of several of the opossums obtained in the autumn of 1912 from New Jersey several features of interest were noted, which may be briefly stated as follows: The thyroid epithelium, instead of being uniform, as is usual in vertebrates, contained, in addition to the usual type of epithelial cells, ovoid cells, parietal in position with reference to the follicles, filled with fine eosinophile granules which gave them a striking resemblance to the oxyphile cells of the anterior lobe of the hypophysis. The epithelium of the thyroid follicles contained large needle-shaped crystals. The thyroid glands of those animals which were kept in the laboratory for two weeks or longer showed a high degree of hyperplasia, associated with the disappearance of the contents of the follicles and of the crystals and, in those kept for a longer period, the appearance of a new secretion antecedent in the form of granules along the free border of the cell.

Since it was possible, considering the time of year at which the collections were made, that the characters and changes in question were associated in some way with the phenomena of hibernation. I made preparations to obtain a larger number of animals, during the past winter, in small groups caught at different parts of the season, and immediately shipped to the laboratory. I have been able to secure these, through the aid of Mr. Elbert Clark, from Waldo, Arkansas. From each of these series, received on October 21, 1913, December 5, 1913, December 22, and January 19, one or more animals were examined immediately, while others were kept for varying lengths of time in the laboratory, and then examined, or were used for experiments as indicated later.

The thyroids of all animals examined immediately after their arrival at the laboratory showed the normal type of gland. There were minor variations in the size of the vesicles and in the shape of epithelial cells, but, in all, the follicles were well formed and spherical and filled with a deeply staining colloid. In all the animals also the ovoid cells mentioned above were present in large numbers, and large crystals occurred in the cells of the follicular epithelium.

The thyroids of the animals examined two or more weeks after their arrival at the laboratory showed a high degree of hyperplasia and cell overgrowth. That hibernation had nothing to do with these changes is indicated by the fact that no change of this sort was apparent in animals taken in midwinter and examined immediately, and that in an animal examined on June first there were but slight evidences of involution of the hyperplastic gland.

The thyroid gland of the opossum consists of two ovoidal lobes situated one on either side of the trachea at the posterior end of the larynx. Usually no isthmus is present but remains of it are seen in the form of minute lobes attached to the posterior end of the main lobe. In some cases this isthmus lobe reaches a considerable size, and in one case a complete isthmus was found. In one case also an accessory thyroid gland was found in a mesial position low down in the neck.

Figure 1 represents a section from a thyroid gland of an animal sacrificed immediately after its arrival at the laboratory. The vesicles in many were larger than in the illustration, the amount of colloid greater and the epithelium flatter. It will be noted that this gland differs from the familiar type of vertebrate thyroid in two features, namely, the presence of a second sort of cell and the presence of large crystalloids in the regular epithelial cells.

The crystals are invariably present in the cells of the thyroid gland examined immediately after capture of the animal, though as will appear later they may be absent or nearly so in the glands during the period of active hyperplasia. They are exclusively intracellular in the epithelium of the vesicles, and never occur in the parietally placed ovoidal cells. Their solubilities have not been accurately determined. Their protein nature is indicated by



Figure 1

the strong Millon's and xanthoproteic reactions which they give in sections. They are visible in the fresh tissue examined in salt solution, but disappear with crossed Nicols. When the animal is injected with an oxazine dye known commercially as "new methylene blue GG," the crystals stain a deep lilac color. The cholesterin reaction, and the reaction for phosphates are negative. The staining with new methylene blue GG in the fresh gland indicates their permeability to this dye, and suggests that notwithstanding their crystalline form they are permeable to other substances in solution.

The ovoid cells resemble anterior lobe cells of the hypophysis. They contain a multitude of tiny granules, easily visible in the fresh cell, though of low refractive index, and staining readily in the living cell with the dye mentioned in the foregoing paragraph. The nucleus is large, oval in outline, located nearer one end of the cell, and richer in chromatin than the nuclei of the regular thyroid



epithelium. In stained preparations, among the granules in the pole of the cell which contains the larger amount of protoplasm, may be seen the delicate network of canals which Holmgren regards as of trophospongial origin but which I consider the homologue of the vacuolar system of plant cells. In these canals is a substance which stains faintly pink in sections of formalin zenker material stained in Mallory's phosphotungstic acid hematoxylin. Sometimes these canals are expanded locally to oval, fusiform, or spherical vacuoles containing the same substance.

These cells are always peripheral in position, and never extend to the lumen of the follicle. They are, however, in immediate contact with the follicular epithelium, and no reticulum extends between the two type of cells. They are distinguished from tissue mast-cells by the fact that the granules of the latter stain pink *intra vitam* with new methylene blue GG, while those of the ovoid cells stain blue. From Unna's plasma cells and from fibroblastic cells they are distinguished by the discreteness of their granulations, by their size and by the fixation properties. The best mode of demonstrating them is fixation in formalin zenker, staining in Mallory's phosphotungstic acid hematoxylin, in which the granules stain deeply blue. In preparations stained with hematoxylin and eosin the granules stain red, and a similar distribution of the acid and basic dyes follows staining with toluidin blue and acid fuchsin. In the preparations so stained, blue stained floccules may be seen distributed through the cell protoplasm, in addition to the small oxyphile granules.

The distribution of the ovoid cells in the thyroid of the opossum is irregular. In three glands serially sectioned they were more abundant in the anterior three-fourths of the gland. The posterior fourth contained few and the isthmus and one accessory thyroid none. That the cells in question are special internal secreting cells there can be little doubt but what their homologues in other vertebrates may be, remains wholly obscure. The possibility that they may represent a dispersed parathyroid has been considered but no proof of this has been obtained. Indeed they resemble the usual cells of the parathyroid glands as little as they do those of the thyroid though some resemblance to the eosin-

ophile cells described in the human thyroid by Welsh and others may be perceived.

As indicated above, the thyroids of all animals examined after two or more weeks in the laboratory show a high degree of hyperplasia and cell overgrowth. In the first month of this process numerous mitoses may be seen in the cells of the thyroid gland, and the latter increase considerably in size. This increase in size is associated with a proportional increase in the quantity of mitochondria and with an increase in size of the individual filaments. Though the process is invariable in the animals kept in captivity there is some variation in the rate at which it proceeds.

Figure 2 represents a follicle from a gland taken from an animal kept in the laboratory for a period of six months. The hyperplasia though high is not materially greater than that observed in two animals from the same group killed during the first month of captivity. Indeed, the results of the observations on all the groups indicate that the hyperplasia proceeds very rapidly at first, then slows down, though mitoses may be found even after six months even in glands which are reverting to normal type after iodine administration. It will be noted in figure 2 that the follicle has expanded to a large complex mass of cells in which the original lumen is still visible though it no longer constitutes a secretion space. Instead, secondary secretion spaces, suggesting an attempt to reconstruct the thyroid by breaking the hyperplastic cell-mass into new independent alveoli, are to be seen. These secondary secretion spaces, not much larger than a red blood corpuscle, contain a small globule of colloid, which stains blue in Jones' modification of Mallory's anilin blue method. The borders of the cells next the lumen contain a few granules, apparently a secretion antecedent. These granules differ from intracellular colloid in their properties inasmuch as they occur always at the extreme border of the cell while the colloid may be deep in the protoplasm of the cells or even external to the nucleus (fig. 3). They are with difficulty preserved and then only in the most peripheral part of the piece of tissue, indicating a different solubility from that of the intracellular colloid. When fixed by formalin zenker or by acetic osmic bichromate they stain readily with neutral gentian and are



Figure 2

stained blue by Mallory's phosphotungstic hamatoxylin. They resemble certain granules which I have found in the hyperplastic thyroid of man but the granules are smaller than in man. Whether these granules represent an incompletely elaborated colloid, or another normal secretion which is exaggerated in the hyperplastic gland or a new secretion, I cannot fully determine. The facts so far, however, are opposed to the first assumption for as will appear later, in the gland which is reverting to normal as the result of iodine administration, and in which colloid secretion is proceeding



at a rapid rate, the latter makes its appearance in the cells in the form of droplets which have unquestionably the characters of the colloid as seen inside the follicle, and this resumption of normal activity is associated with a disappearance of the granules described above. I am inclined therefore to the view that the granules represent a new secretory product or a normal secretory product different from thyreoglobulin, which is not present in the normally secreting gland in sufficient quantities for microscopic detection. In either event the secretory condition of the hyperplastic gland would represent a perverted secretion indicated either by the introduction of new secretory products or the disturbed equilibrium of normal products. In these hyperplastic glands, here and there, but very rarely, small droplets of colloid may be seen in the protoplasm of the cells. They are usually more deeply placed in the cell than the granules discussed above and show the characteristic staining properties of intrafollicular colloid.

The ovoid cells apparently share in the hyperplasia for in the hyperplastic glands they are much more numerous than in the normal glands. In some cases large groups of cells differing in some respects from both types, but which I take to be the result of hyperplasia of the ovoid type, are found. These cells stain deeply blue in Mallory's phosphotungstic acid hematoxylin but the proplasm is much reduced in comparison with the normal oval cells, and the definite granulation is not seen. In these groups also mitoses may be seen.

The degree and rate of hyperplasia is subject, in different animals, to some variation, but some degree of hyperplasia is invariable in the animals kept in captivity. In some animals the degree of hyperplasia is such that the whole thyroid gland is converted into a continuous complex of branching and anastomosing epithelial cords, which give it a superficial resemblance to the parathyroid gland.

In the intermediate stages of this hyperplasia some glands show a complete absence of colloid, most of them show a great reduction of colloid and, in the earlier phases, few of the border granules described above. The crystals also disappear or become greatly

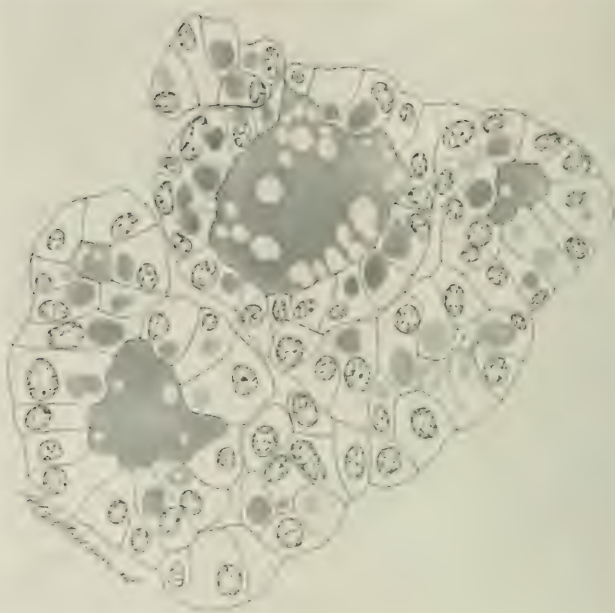


Figure 3

reduced in size and in number. Since colloid originally present in large amount rapidly disappears from the gland and since the evidences of normal colloid production are almost wholly lacking (note the absence of intracellular colloid as well as intrafollicular colloid) and since the resumption of activity after the administration of iodine is marked by the appearance of colloid both in the cells and in the follicles, in my opinion the conclusion, which is also probable on general biological grounds, that this gland in the phase of active hyperplasia and cell overgrowth has a low secretory rate and potential, is justified. On the other hand, after the period of most active hyperplasia is past the gland resumes activity though of an abnormal sort, marked by a low rate of colloid production and of crystal production and the appearance of a new secretory antecedent in the cells.

Since I wished to establish the independence of the hyperplasia of captivity with reference to the phenomena of hibernation the

number of animals available for experiment has been small. I hope to return to this aspect of the question next year for the readiness with which this animal's thyroid undergoes overgrowth in captivity suggests the possibility of controlling both hyperplasia and reversion experimentally. The experiments on feeding gave no definite results though the border granules were more abundant in an animal kept for two months on meat and egg diet exclusively, as compared with several animals kept for a similar length of time on a mixed diet of meat, bread, and apples.

With regard to the effect of iodine administration one experiment made in the autumn suggests the possibility that there is a refractory period, in regard to iodine, at the height of hyperplastic activity. This animal, a female received October 21, was made the subject of a hemilobectomy on November 18. The gland removed showed a high degree of hyperplasia. For two weeks the animal received a dose of 5 drops of syrup of iodide of iron daily, and, at the end of the time, the other lobe was removed and examined. No substantial change was noted in the second lobe.

On the contrary, experiments made in the spring on animals which had been present in the laboratory all winter and in which as the controls showed there was still a high degree of hyperplasia and but little tendency to reversion gave a prompt and characteristic reaction to iodine. On May 8 four animals which remained in my collection were set aside for iodine experiments. Of these two were retained for control and to each of the others was administered daily 5 drops of syrup of iodide of iron. One of the controls died and was unavailable for examination. One of the iodide animals was killed after 17 days, the other after 24 days. The remaining control animal was thyroidectomised on the same day as the last iodine animal and the thyroids fixed for histological examination.

Both of the animals which received iodide showed an advanced degree of colloid involution of the thyroid gland, and this was more advanced in the twenty-four-day animal than in the seventeen-day one. In both practically every cell in the thyroid gland contained one or more droplets of colloid, and in each the reformation of the follicles had advanced to a degree which was pro-



portional to the duration of the experiment. The control gland differed in no respect from the hyperplastic glands examined earlier in the year, that is, showed practically no reversion. Figure 3 shows a section of the thyroid from the twenty-four-days iodide animal. In this gland it is to be noted that there was no increase in the number of intercellular crystals as compared with the control gland. The resumption of colloid activity was a common property of the thyroid epithelium and was not associated with the formation of the so-called colloid cells of Langendorff. The border granules disappeared from the cells with the resumption of colloid activity.

These observations on the opossum establish in my opinion by study of both the hyperplasia and involution the high degree of lability and rapidity of reaction in the thyroid gland, on which Marine and his co-workers have long insisted. They furnish an opportunity to control and to analyze the factors involved in thyroid hyperplasia. They confirm Marine's conclusion of a low rate of colloid production in the hyperplastic gland. In addition, by demonstrating a new type of cell in the thyroid gland of the opossum and a new secretory product in the cells, they furnish objective evidence of that polyvalency of thyroid secretion which has been so often postulated in the discussion of morbid conditions of the gland.

## COVERS FOR DISSECTING TABLES

T. WINGATE TODD

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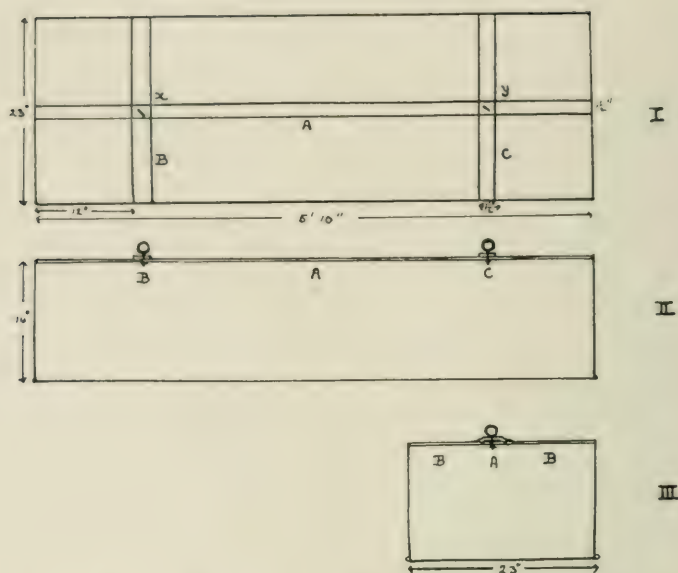
### THREE FIGURES

In order that the border-line areas between the different 'parts' of a cadaver may be studied efficiently by the student, it is essential that dissection of the whole subject be carried out before dismemberment. In this way alone is it possible to obtain a correct impression of the vagus system, of the relations and distribution of the limb plexuses, of such muscles as the psoas and the obturators and of many other points in human anatomy. But the greatest technical difficulty in attempting this lies in the fact that the cadavers dry out so easily in spite of every care. For the student to be able to revise his work from time to time, it is essential that the cadaver be kept in the best possible condition for six months or even more, during which time, of course, dissection is proceeding every day. The utmost care leaves much to be desired in this respect because it is impossible, even were it desirable, to move the cadaver into a tank or store each day when dissection is finished, and no subject kept continuously on the dissecting-room table can long remain in fresh condition even with the precautionary measures of plenty of cloths and waterproof covers in addition to repeated moistening with the various fluids in vogue for such a purpose.

Our final attempt at Western Reserve University to overcome the difficulty of adequately preserving cadavera in the dissecting-room has met with such success that it may perhaps be a useful suggestion to other laboratories where the same difficulties are to be met.

Each table is mounted on castors so that it may be easily moved, and is provided with a galvanized iron cover, which can be drawn to the ceiling when work is in progress. The cover is made of number 20 galvanized sheet iron. Its dimensions are: length, 5 feet 10 inches;<sup>1</sup> breadth, 23 inches; depth, 14 inches. It is made watertight. Its lower margin is flanged round a steel wire to give it greater rigidity and finish. Its top is strengthened by three strips of iron  $\frac{1}{4}$  inch in thickness and  $1\frac{1}{2}$  inches in breadth. These are riveted to the top, as shown in figures 1 to 3, and at each intersection an iron ring (2 inches in diam-

<sup>1</sup> The length had to be 5 feet, 10 inches, to fit our tables. A 6-foot length is desirable, but we find that with our routine measure of keeping the feet at right angles to the legs when the cadavera are embalmed, there is no difficulty in getting the cover to fit over all ordinary subjects.



Figs. 1 to 3 Plans for the making of the covers; scale one-half inch to one foot. Figure 1 represents the plan of the roof of the cover; figures 2 and 3 correspond to side and end elevations respectively.

eter) is bolted to the cover. The cover fits accurately on to the table, and can easily be raised or lowered at will by means of cords and pulleys fixed to the ceiling. Parallel with the suspension pulleys, but about 3 feet distant from them, two 100-candle-power Mazda lamps are suspended, so that when the cover has been raised the table can be drawn from a position directly under the cover to one beneath the electric lights. At first it was thought that when the cover was raised any contained condensed moisture might drip from the inside, but it has been found that there is not moisture enough to cause any trouble. The covers are painted white both within and without, and thus add to the clean and tidy appearance of the room, while they do not interfere with the lighting when they are raised.

In addition to the efficient preservation of the cadavera, the covers keep the tables free from dust and therefore prevent any soiling of dissections placed on the tables. Moreover, they add to the neatness and well-being of the dissecting-room, and transform it, so far as the members of other departments of the University are concerned, from a gruesome, somewhat repulsive apartment into a clean and pleasing laboratory. Certainly the appearance of covered tables is much more agreeable than that of tables on which the outlines of the cadavera are plainly suggested under the folds of the dark-colored waterproof covering.



The practical application of the idea was carried out by Prosector Leonhart, and the students were quick to recognize the advantages of the covers so that no 'regulations' are necessary for their use.

One last consideration may be mentioned in favor of such covers as are herein described. Should they become obsolete for their present purpose, their size, their watertight build and their strengthened top allow them to be turned upside down and used as tanks for the preservation of material. In case of such use the removal of the iron rings leaves the tank with two holes in the bottom into which can be fitted ordinary corks, and which provide for drainage of fluid and efficient cleaning.

## A TANK FOR THE PRESERVATION OF ANATOMICAL MATERIAL

T. WINGATE TODD

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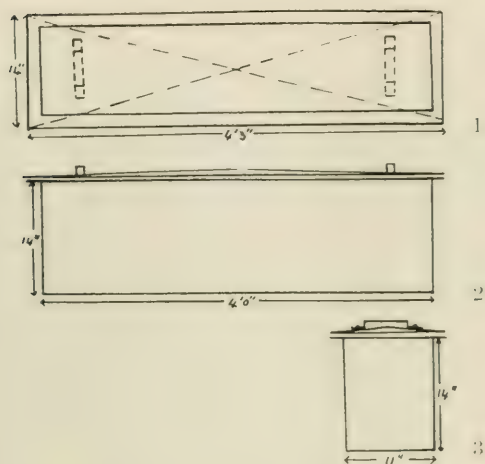
THREE FIGURES

The adequate preservation of gross anatomical material requires some form of receptacle or tank, and if large quantities of material or dissections either of human or mammalian anatomy are to be carefully kept in good condition, it is necessary that the receptacles be so cheap as to be readily multiplied with the growing needs of the department. In Western Reserve University we have, besides the dissecting-room, a museum, material for which accumulates faster than it can be mounted for exhibition, under present circumstances. In addition, arrangements with the various hospitals and with the city administration result in the acquisition of much fetal material and the bodies of all animals from the Zoölogical Gardens. For these reasons it has been necessary to provide such accommodations as shall be at once cheap and serviceable.

The form of tank described below has fulfilled these requirements, and is therefore now being used in other laboratories. Hence it seemed advisable to make a record of it as one more laboratory furnishing suitable for anatomical departments.

The tank, the plans for the manufacture of which are also submitted with this communication, is made of galvanized iron, number 20 thickness. It is watertight, and provided with a flange running round its upper margin, the flange being  $1\frac{1}{2}$  inches broad. The lid is simply a sheet of the same metal slightly scored diagonally from corner to corner so that a somewhat concave surface is presented to the contents and the flow of the condensed fluid which accumulates on the under surface of the lid directed to the corners. In order to seal the tank hermetically, the flange is thickly smeared with vaseline.

The thickness of the iron is found to be sufficient to prevent undue bending of the flange, but of course the result of any accident to flange or lid can readily be repaired with the hammer. The vaseline method of sealing has proved equally efficient with the method whereby the lid is made to fit into a channel filled with glycerine round the top sides of the tank, and is much more convenient than any other scheme of tank lid. The inner surfaces of tank and lid are coated with asphaltum, which is renewed from time to time. The tank is cheap, tight, portable, does not get out of order, and is very easily opened, closed or cleaned.



Figs. 1 to 3. Plan of tank measuring  $48 \times 11 \times 14$  inches; scale one-half inch to one foot. Figure 1 shows the plan of the tank, with the lid in dotted lines. Figures 2 and 3 represent side and end elevations.

It has proved much easier to use and more convenient than the usual form of tank made of slate, stoneware, wood or lead-lined wood. It can always be made locally, and the stock can be increased at very short notice. It can be made of any size up to one which will hold the larger Mammalia. But it is well to have a plug in the floor of the bigger tanks so that they may be emptied of fluid and cleaned more readily. The idea originated in a somewhat similar tank in use for the preparation of color specimens by the Kaiserling method in the Pathological Laboratories of the University of Manchester.

It is convenient for storing purposes to have standard sizes, and the dimensions which have been found most useful by Mr. Leonhart, Prosector to the Department, are the following:

DIMENSIONS IN INCHES			
	Length	Breadth	Depth
Kaiserling preparation.....	24	11	8
Brain storage.....	48	11	8
Limbs or pelves.....	48	11	14
Torsos.....	48	22	14



It is obvious that any size may be made; only those which are most generally useful have been detailed. If it is desired to suspend the brains in fluid from rods placed across the tank, plaster slabs 1 inch in thickness may be made to fit the length of the tank. Grooves may then be gouged out of the upper margin to accommodate the rods. Finally, the plaster slabs may be rendered hard by boiling them in oil, one of the brain tanks being used temporarily for this purpose.

## A SIMPLE ELECTRICAL HEATING DEVICE FOR INCUBATORS, ETC.

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### FOUR FIGURES

The advantages of electricity as a means of heating incubators, paraffine baths, etc., are, I believe, everywhere recognized. Gas, with all its uncertainty and inconvenience, is still employed in many laboratories on account of the prohibitive cost of the various high-priced electric incubators on the market. Many excellent devices have been designed by laboratory workers, and described in this and other journals, for the utilization of electricity for heating incubators originally designed for gas, and the most of these work very satisfactorily where direct current is available in the laboratory. The fact that there may be other laboratories confronted with the same problems that we have here has prompted me to offer the suggestions contained in this paper. Our laboratories are at present supplied with 110 volt alternating current only, and a lack of mechanical facilities suggested the modification of the old forms of gas regulators for use with electricity.

The heating element made use of was made of nichrome wire. About 40 feet of number 18 nichrome wire was wound, on a lathe, into coils of  $\frac{1}{4}$ -inch inside diameter, and the entire coil stretched between pegs arranged in a 'transite' base the size of the incubator. A wooden frame, lined also with transite board, was constructed so as to support the incubator about  $\frac{1}{4}$  inch above the coils. The heating chamber thus formed was completely insulated on all sides with transite. Copper leads connected the ends of the heating coils with binding posts on the outside.

For thermo-regulators I have been able to modify several forms of gas regulators so as to operate a "make and break" device. Two examples will suffice. The mercury-glass form of regulator, such as the Novy or Reichert is modified by sealing a platinum wire (*a*, fig. 1) into the tip of the inner regulating tube and attaching another to the regulating screw (*d*). When the mercury rises to the point (*f*) a contact is made, and when the mercury falls, due to cooling, the contact is broken.

The Roux bimetallic regulator may be utilized as follows: The hole at (*x*, fig. 2) should be reamed out and the upper part (*c*, *d*, *e*), insulated from the arm (*y*) by means of washers of mica or other non-conducting

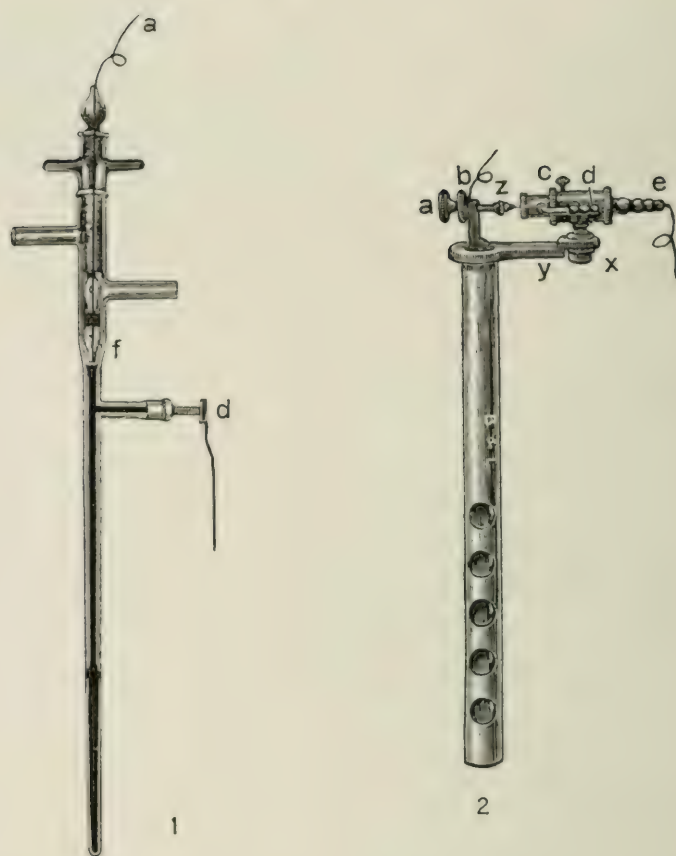


FIG. 1 A modified Reichert regulator; *a*, platinum terminal sealed into inside tube; *d*, terminal fastened to regulating screw; *f*, point of contact between platinum terminal and mercury.

FIG. 2<sup>1</sup> A modified Roux regulator; *a*, regulating screw; *b*, lock nut with terminal attached; *c*, terminal soldered to gas tube; *x*, insulated joint; *y*, regulator arm; *z*, point of contact.

material. Wires are then attached at (*b*) and (*e*), the circuit being made and broken at (*z*). In this case the circuit is made when the bimetallic part of the regulator is cooled below the desired temperature. Therefore, with this regulator, the connections should be arranged so that the current will flow through the heating element when the cir-

<sup>1</sup>These figures have been reproduced by courtesy of Bausch and Lomb Optical Co.



cuit through the regulator is closed. When the mercury-glass form is used the opposite should be the case. Many of the other forms of gas regulator may be modified in a similar manner, at a very small cost and with very little work.

These devices may be used with either direct or alternating current, but the arrangement will be somewhat different in the two cases. With either type of current the actual making and breaking of the heating current is accomplished by means of an ordinary telegraphic relay, to prevent arcing at the points of contact in the regulators, through which only a very small current can be allowed to pass. When direct current is used, the regulator and electromagnet of the relay together with a large resistance ( $R$ ) are connected in parallel with the heating element (fig. 3). The resistance is very large so as to allow just enough current to pass through the regulator to actuate the relay, and no more.

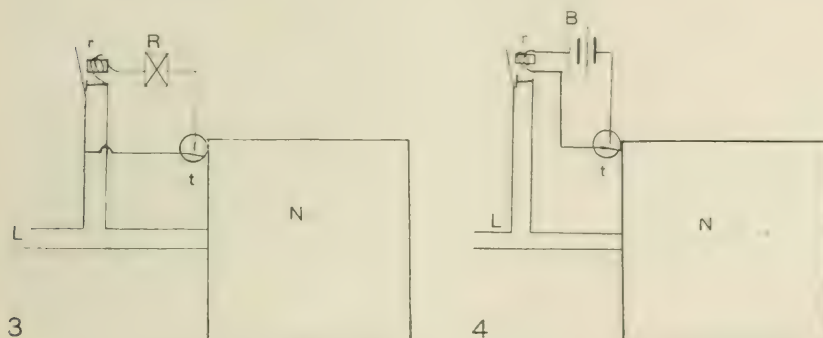


FIG. 3 Diagram of connections for direct current;  $N$ , heating element;  $L$ , electric line;  $t$ , regulator;  $r$ , relay;  $R$ , resistance.

FIG. 4 Diagram of connections for alternating current;  $B$ , battery; otherwise lettered the same as figure 3.

When alternating current is used, other means must be employed to operate the relay. In this case the manner of connecting is as shown in figure 4. To supply the regulating current here I use a battery of three Columbia dry cells, with a 250 Ohm. telegraphic relay. As used here the cells require renewing about once in four months. Within that time there is absolutely no danger of the relay failing to operate, in fact in some cases the same battery has been used for a much longer time. With any of the devices mentioned temperature regulation is much more accurate than would be supposed. The variations, on a scale extending from room temperature to a point  $70^{\circ}\text{C}$ . above room temperature, is always less than one degree, which is accurate enough for all routine zoölogical work, and much more accurate than the ordinary gas regulator, especially if the gas pressure is somewhat variable.

## NOTICE

The next annual meeting of the American Association of Anatomists will be held in St. Louis during December. The Association goes to St. Louis as the guest of Washington University. The exact dates of the scientific sessions will be announced later.

# THE DEVELOPMENT OF THE ADRENAL GLANDS OF BIRDS

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*From the Laboratories of Animal Biology of the State University of Iowa*

EIGHT FIGURES

## CONTENTS

Introduction.....	451
Observations.....	456
Early development of cortical substance.....	456
Early development of chromaffin substance.....	461
Development after 216 hours.....	464
Development of the venous system.....	465
Development of the arterial system.....	469
The glands of the adult bird.....	471
Summary.....	472
Bibliography.....	473

## INTRODUCTION

Although the development of the adrenal glands has been studied for the various classes of vertebrates for a number of years and by many investigators of recognized ability, there seems to be no very general agreement in their conclusions; and several opposing theories have been developed as a result. This is especially true of the observations on the development of the adrenals of birds. Here the field is in a most chaotic condition and a review of the literature shows, that while one theory may have the weight of evidence in its favor, each of them is supported by a number of investigators whose ability is of the highest order. No minute description of the development of the vascular system of the adrenal glands of birds has as yet appeared. The development of the vascular system of the adrenal glands of mammals has been reported; but this cannot be taken as a



criterion for the development in birds, since in birds there can be no sharp division of the glands into cortex and medulla.

The object of this investigation is to determine the source and manner of development of the various systems of the adrenal glands of birds, and to make clear the relationship existing between these different systems in the birds.

It is safe to say that there is no longer any doubt as to the nature of the adrenal glands, since the fact is well established that in the higher vertebrates they represent the more or less complete union of the interrenals and suprarenals of the lower vertebrates. The adrenal glands of the higher vertebrates are then a pair of organs, each representing an interrenal and a suprarenal gland of the lower classes of vertebrates. In the adrenals of mammals, the cortical substance represents the interrenals, while the medullary substance corresponds to the suprarenal glands of the lower vertebrates. Since in the case of birds there is no true medulla, the term 'chromaffin substance' will be used in place of the term 'medullary substance.'

According to the different investigators, the cortical substance has been derived from several possible sources; the mesenchyme, the mesonephros, the germinal epithelium, the peritoneal epithelium, and the sympathetic ganglia.

Gottschau ('83) and Minot ('97) took the view that the cortical substance develops from the mesenchyme, the former working with mammalian embryos and the latter with human embryos. The theory of mesonephric origin was supported by Semon ('87) and C. K. Hoffmann ('92), both working with the embryos of birds. Von Mihaleovics ('85) working with reptiles, and Janošik ('83, '90), Fusari ('93), and Loisel ('04), working with bird embryos, found a very intimate relationship between the adrenals and the genital glands and took the view that the adrenals develop from the germinal epithelium, so far as the cortical substance is concerned. O. Schultze ('97), from his observations made on embryos of *Vespertilio murinus*, concluded that the cortical substance of the adrenal gland arises from the sympathetic ganglia. The following observers support the theory that the cortical substance of the adrenal glands develops from the

peritoneal epithelium. Valenti ('93) and Souli ('03), working with bird embryos, and Kuntz ('12), working with embryos of *Thalassochelys caretta*. This view is also supported by Poll ('06). The above citations do not cover the entire field but are given only to show the various theories which have been proposed to account for the cortical substance of the adrenal glands.

Several theories have also been proposed to account for the development of the chromaffin substance of the glands. Here again there is a lack of agreement in the conclusions of the various investigators, as was the case, in the observations on the development of the cortical substance. Gottschau ('83) and Minot ('97) derived the chromaffin substance from the mesenchyme, the former from observations made on mammalian embryos and the latter, from human embryos. Von Mihalecovics ('85), from observations made on reptilian embryos, came to the conclusion that the chromaffin substance of the glands is derived from the germinal epithelium. This theory was upheld by Janosik ('83, '90) and Valenti ('89, '93), both working with embryos of birds. Leydig ('53) described the interrenals and suprarenals of fishes and came to the conclusion that the suprarenals are derived from the sympathetic nervous system. Balfour ('78) in his classical work on the elasmobranch fishes, shows conclusively that the suprarenals are derived from the sympathetic ganglia along the abdominal aorta. Since that time many investigations have verified these conclusions and it is hard to account for the fact that many of the earlier investigators refused to accept the results of the work of Leydig and Balfour. Among the later investigators to hold the theory of sympathetic origin of the chromaffin substance are: Fusari ('90, '93), H. Rabl ('91), Minervini ('04), and Loisel ('04). These investigators all worked with bird embryos. Souli ('03) and C. K. Hoffmann, ('89, '92), working with the embryos of birds and reptiles, came to the theory of sympathetic origin. This theory was also supported by the work of Poll ('06) in which he used the embryos of mammals, reptiles, and birds. Kuntz ('12), from observations made on the embryos of *Thalassochelys caretta*, concludes that the chromaffin substance develops from the analgen of the

prevertebral sympathetic plexuses. The above citations, while they do not cover the entire field, serve to show the confusion which exists concerning the development of the adrenal glands. A complete bibliography will be found in the work of Poll ('06).

There are two general theories to account for the origin of the cortical and chromaffin substances of the adrenal glands: the theory of homogeneous origin and that of heterogeneous origin. The supporters of the theory of homogeneous origin have in turn derived the adrenal glands from the sympathetic nervous system, from the mesenchyme, and from the germinal epithelium. There is the same lack of agreement among the supporters of the theory of heterogeneous origin. These investigators have in turn derived the glands from the mesonephros and the peripheral part of the sympathetic nervous system, the germinal epithelium and the sympathetic nervous system, and from the peritoneal epithelium and the sympathetic nervous system. Poll, from extensive observations and from a thorough review of the literature, shows that the weight of evidence favors the theory that the cortical substance of the adrenal glands of all vertebrates is derived from the peritoneal epithelium and that the chromaffin substance develops from the cells which break away from the anlagen of the peripheral part of the sympathetic nervous system.

Very little work has been done on the development of the blood vessels of the adrenal glands. Flint ('00) has worked out the blood vessels of the adrenals of mammals and reports a very interesting condition existing in this class of animals, especially as regards the venous circulation. According to this investigator the venous system may be compared to a tree, the terminal twigs uniting to form larger branches and as a natural result of this process a large central vein is formed. He found that in most cases this central vein opens into the postcava as a single vein. In the dog, however, the central veins of the posterior and anterior lobes of the gland do not unite, but open into the postcava separately. This description refers only to the venous system of the medullary part of the gland. The venous system of the cortical part of the gland is of no great importance, being



composed of the terminal twigs of the medullary venous tree. The arteries of the gland, according to Flint, are derived from five sources: *A. phrenica*, *A. phrenica accessorius*, *A. lumbalis*, *A. renalis*, and the abdominal aorta. These arteries branch out on the capsule of the gland forming a network of blood vessels over the entire gland. These branches finally enter the cortex at various points and break up into capillaries, the terminal branches penetrating the medulla for a short distance.

Miller ('03), working on the development of the postcaval vein in birds, did not make any attempt to work out the development of the veins in the adrenal glands other than to determine their origin. He concluded that the veins of the left adrenal develop from the subcardinal vein and probably those of the right gland are of the same origin.

Minot ('00) distinguishes between capillaries and the venous blood vessels found in several organs of the vertebrates, among these being the adrenal gland. He finds these blood vessels differing from capillaries in size, shape, relation to other tissues, and in their method of development. According to this author, these blood vessels which he calls sinusoids are larger than capillaries and are irregular in section. The walls of sinusoids are composed of a single layer of endothelial cells resting upon the parenchyma of the organ, while a capillary always has a connective tissue wall upon which the endothelial layer rests. The manner of development also differs, the capillaries developing from a chain of vasoformative cells which becomes hollowed out and connected with a vessel already formed, while sinusoids develop by the outpushing of the endothelium of the wall of a pre-existing blood vessel.

The vascular spaces observed and described by Kuntz ('12) in the adrenals of *Thalassochyles caretta* are undoubtedly identical with the sinusoids of Minot. Flint ('00) finds no sinusoids in the adrenals of mammals and is of the opinion that the investigators who have reported them used sections which were too thin to show the true structure of the walls of the blood vessels.

The following observations are based exclusively on embryos and adult of the domestic fowl (*Gallus domesticus*). All speci-

mens were fixed with chrom-aceto-formaldehyde and stained by the iron hematoxylin method. Embryos were injected with india ink after the method of Knowler ('08). The adults were injected with a gelatin mass. Sections used for the study of the development of the vascular system were cut to a thickness of 20 micra. All other sections were 10 micra thick.

It gives me great pleasure to express my indebtedness to Prof. F. A. Stromsten for many helpful suggestions during my investigation of this subject and also for reading the manuscript. I take the greatest pleasure in acknowledging my indebtedness to Prof. G. L. Houser for suggesting the subject of this investigation and for many helpful suggestions during its progress.

#### OBSERVATIONS

##### *The early development of the cortical substance*

The cells which are later to form the cortical substance of the adrenal glands of birds are first seen in the 96th hour of incubation. They appear as a thickening of the peritoneal epithelium, ventral and mesial to the mesonephros, ventral to the abdominal aorta, and dorsal to the hind gut which is open at this time (fig. 1, *ad.*). The developing cells push in dorsally from the epithelium upon which they rest and become larger and more nearly circular in outline than those cells from which they arose, that is, the cells of the peritoneal epithelium. The nuclei are correspondingly enlarged and mitotic figures may be seen in nearly all of them. The nuclei also differ from those of the parent cells in their staining properties, these nuclei all being less deeply stained and less granular than those of the peritoneal epithelium. It is probably due to the fact that the anlagen of the cortical substance appear so early in the development of the chick, that earlier investigators, using embryos which had passed this stage of development, derived the cortical substance from other sources.

During this early period of incubation the development of the cortical substance goes on with astonishing rapidity, and nine hours later, during the 105th hour of incubation, the cortical

cells have piled up on the peritoneal epithelium so that a solid body is formed on each side of the base of the mesentery. In the meantime, folds have appeared in the peritoneal epithelium which throw these cell groups further from the base of the mesentery,

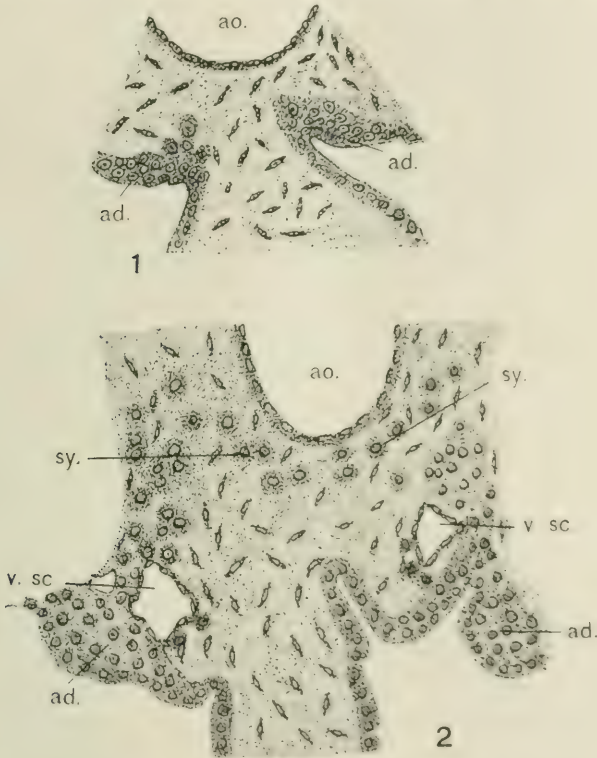


Fig. 1 Transverse section through the adrenal region of a 96-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta.  $\times 130$ .

Fig. 2 Transverse section through the adrenal region of a 105-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein.  $\times 130$ .

laterally. At this period they lie just mesial to the ventral side of the mesonephros. This is possible because the mesonephros lies closer to the median line than in the preceding stage, due chiefly to the fact that it has been growing rapidly during this period. The character of the cortical cells has not changed



during this period, but their migration has gone on rapidly until a chain of cells can be traced from the group resting on the peritoneal epithelium, to a point just slightly dorsal to the ventral level of the aorta (fig. 2, *ad.*). These cells then lie between the aorta and the mesonephros, in the mesenchyme. In this migration most of the cells pass laterally to the subcardinal veins but this does not hold true for all of them, since a few of them take a path median to these veins. The shape of these cells and their nuclei, together with their staining properties, make them easily identified and the course of their migration can be followed without difficulty.

During the next fifteen hours, or after 120 hours of incubation, the cortical cells have become detached from the peritoneal epithelium and all of them have migrated dorsally. At this stage of development they appear as scattered cell groups reaching from the dorsal level of the subcardinal veins to the middle level of the aorta. They have reached about the same level on both the right and left sides of the aorta, though those on the right side may be slightly in advance of those on the left. These cell groups are scattered through the mesenchyme between the mesonephros and the aorta and have practically invaded the entire region. The nuclei are still circular in section and show well developed mitotic figures. Occasionally cells may be seen undergoing division. Isolated cells are still circular in outline but those which are found in groups have become more or less flattened by contact with the other cells of the group and present an oval outline. They may still be identified from anything which has yet appeared by their nuclei and staining properties (fig. 3, *ad.*). The relative position of the cortical cells at this period is shown by figure 6. It is seen that they lie on the dorsal side of the subcardinal veins, lateral and ventral to the aorta, and mesial and ventral to the postcardinal veins. The region which they occupy extends posteriorly to a point about level with the anastomosis of the subcardinal veins in the median line, ventral to the dorsal aorta.

From the 120th to the 130th hours of incubation there is a great increase in the mass of the cortical substance. This is

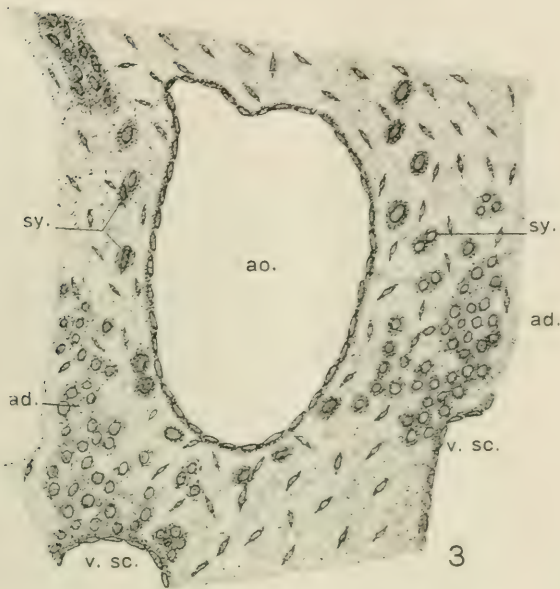


Fig. 3 Transverse section through the adrenal region of a 120-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein.  $\times 90$ .

Fig. 4 Transverse section through the adrenal region of a 130-hour chick embryo; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *ch. a.*, anlagen of the chromaffin substance; *mes.*, mesonephros; *sy.*, anlagen of the prevertebral sympathetic plexuses; *v. sc.*, subcardinal vein.  $\times 90$ .

due, partly to the fact that the cells are no longer scattered through the mesenchyme, but have collected in large groups, and partly to the fact that the number of these cells has been increased by the division of the older cells present in this region. At this stage the cells are arranged in large solid groups lying dorso-mesial to the subcardinal veins and ventral to the mesonephric arteries which run over the anterior ends of these cell groups. These cells then occupy the region between the aorta and the mesonephros in the region outlined above. Owing to the close arrangement of the cells, they are losing their regular shape, but the nuclei remain circular in outline and continued development is shown by the presence of mitotic figures (fig. 4, *ad.*).

After 144 hours incubation the cells have become more closely grouped than in the preceding stages and are found in large oval masses on each side of the aorta. The nuclei have become more granular but still contain mitotic figures. The cells are becoming more irregular in outline and, on account of the proximity of these cells to the mesonephros, and on account of the close resemblance between them at this time, it is difficult to distinguish one from the other. Such conditions, doubtless, are responsible for the conclusions of some of the earlier investigators that the cortical substance of the adrenals is derived from the mesonephros. Careful investigation reveals a thin layer of flattened mesenchyme cells between these two bodies.

Twenty-four hours later, during the 168th hour of incubation, the mass of the cortical substance has greatly increased. The cells have arranged themselves in irregular chains and have taken a roughly hexagonal shape. The nuclei stain much darker than previously but they still show mitotic figures in great numbers, showing that the cortical substance of the gland is still increasing by division of its own cells. The mass of cortical substance is roughly circular in section at this time and occupies practically the same level as the aorta and has about the same cross sectional area through the center. The mesonephros has developed ventrally until it is in contact with the adrenal only at its dorso-mesial angle. The subcardinal veins still lie on the ventral



border of the glands. At this period of development, connective tissue fibers are collecting around the gland, giving promise of a connective tissue capsule later. A few of the fibers are seen within the body of the gland, between the cords of cells.

The cortical substance continues to grow rapidly during the next twenty-four hours and after 192 hours of incubation its cross sectional area is fully twice as great through the center as that of the aorta. The gland is about 2 mm. long at this period. The cell mass is becoming less dense than it has been for some time. A large number of the nuclei still show mitotic figures but in many of the cells these figures are no longer present. At this time, blood cells may be seen in the relatively large openings between the cords of cortical cells.

Little change in the form and size of the gland is seen during the next twenty-four hours. The greatest changes are seen in the internal arrangement of the cells. The gland has become much more vascular during this period and many more spaces have appeared between the cords. The cell cords have become very dense and compact, making it difficult to see the outline of the individual cell.

The above observations lead to but one conclusion, namely, that the anlagen of the cortical substance of the adrenal glands arise as groups of cells which proliferate from the peritoneal epithelium.

#### *Early development of the chromaffin substance*

The observations on the development of the adrenal gland show that the anlagen of the cortical substance arise from the peritoneal epithelium. Observations on the origin of the chromaffin substance seem to show that it arises, not from the same source as the cortical substance, but from the anlagen of the prevertebral sympathetic plexuses. It is evident then that the adrenal glands arise from two separate germ layers, namely, the mesoderm and the ectoderm.

After 120 hours of incubation, large oval cells are seen migrating ventrally from the sympathetic trunks on each side of the aorta. These cells migrate singly in most cases and most of

them pass around to the ventral side of the aorta and later form the prevertebral sympathetic plexuses (fig. 3, *sy.*). At this stage of development the anlagen of the cortical substance are a loose group of cells on each side of the aorta. The cells of sympathetic origin migrate in a path which causes them to pass between the aorta and the groups of cortical cells. At this time there is no connection between these two kinds of cells. The two kinds of cells, cortical and sympathetic, are easily distinguished from one another by their size and affinity for stains, the latter being the larger and taking the deeper stain.

The first evidence of any connection between the anlagen of the prevertebral sympathetic plexuses and the chromaffin substance is seen after 130 hours of incubation. The cortical cells have arranged themselves in large, compact masses by this time and have taken a definite outline. At this time, some of the cells migrating from the sympathetic trunks turn off ventrally in the region of the adrenals and either enter them, or become attached to the surface of the cell groups. Figure 4 (*sy.*) shows several of these cells on the inner edges of the groups of cortical cells, and on the right, one cell may be seen which has penetrated to the center of the cortical substance. This development continues for some time and these new elements, the cells of sympathetic origin, do not seem to differ in any way from those which pass on to form the prevertebral sympathetic plexuses. These cells, then, are indifferent in nature. As the growth of the embryo goes on, more of these cells are found entering the cortical substance of the gland and collecting, in most cases, in groups of two or three. Single cells, however, are found scattered throughout the cortical substance. During this period they may be found almost anywhere within the cortical substance and a great many are found around the surface of the glands.

After 168 hours of incubation, the cells which are to form the chromaffin part of the gland are beginning to show some differentiation. Those which have entered the cortical substance are no longer large circular cells with round, clear nuclei. The shape is becoming irregular, as a general rule, and the cells are smaller than originally. The nuclei are oval and have become quite

granular, in many cases, even more so than those of the cortical cells. They are most easily distinguished from the latter cells by means of their staining properties, both nucleus and cytoplasm taking a deeper blue color with the iron hematoxylin method. These invading cells, at this stage of development, show a tendency to arrange themselves in cords throughout the cortical substance, though many solitary cells are also found. This seems to be the height of the migration of the cells from the sympathetic trunks and at this time the mesenchyme around the glands is shot full of them, and they can be seen entering the glands from all sides.

During the next twenty-four hours, the chromaffin cells within the glands have increased greatly in number and most of the sympathetic cells have disappeared from the mesenchyme around the glands. At this time the chromaffin cells are arranged in cords, many of which have pushed in close to the venous blood vessels. This location with regard to the venous circulation cannot be taken as a general rule at this period of development, since a great number of these cords do not seem to bear any relation to the blood vessels.

The arrangement of the chromaffin cells undergoes a marked change during the next twenty-four hours, 216 hours' incubation. The cells were first found scattered, either singly, or in small groups, throughout the cortical substance. Later they became arranged in cords or columns. At this period the cells cords break down, but do not return to the original condition of solitary cells scattered throughout the cortical substance. Instead of this scattered arrangement, the cells are found in small groups arranged around the venous blood vessels. Of course, not all of the groups are so situated, since the cords from which they originated were not all in contact with the blood vessels.

These observations bear out the contention that the chromaffin substance of the glands does not arise from the mesenchyme or germinal epithelium, but from the anlagen of the prevertebral sympathetic plexuses. These cells enter the cortical substance as indifferent cells and later become differentiated to form the chromaffin substance of the glands.



*Development after 216 hours' incubation*

After 216 hours' incubation the characteristic features of the adrenal glands are firmly established and the development of the glands from that time up to hatching is chiefly one of growth so far as the cortical and chromaffin cells are concerned. The glands increase in volume slowly and become more vascular until, at the end of the period of incubation, they have the

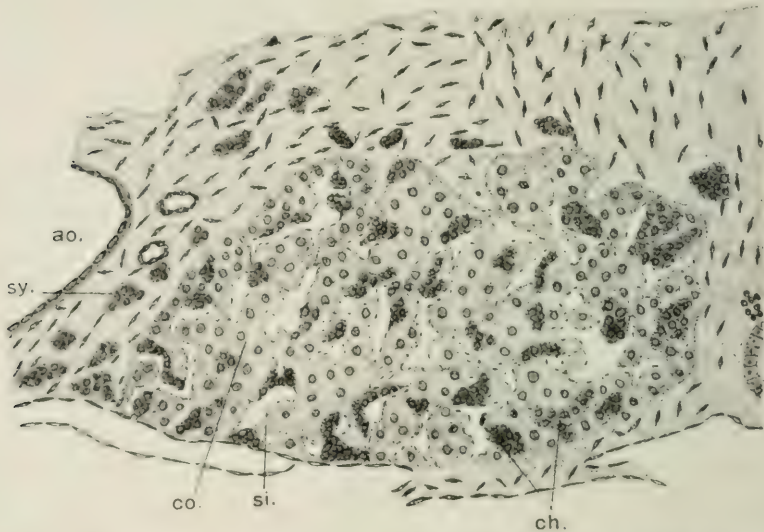


Fig. 5 Transverse section through the adrenal gland of a 264-hour chick embryo; *ao.*, aorta; *ch.*, chromaffin substance; *co.*, cortical substance; *si.*, sinusoids; *sy.*, anlagen of the prevertebral sympathetic plexuses.  $\times 90$ .

appearance, in section, of a large number of groups of cells almost surrounded by blood vessels.

An idea of the structure of the gland may be had by referring to figure 5. Here the gland is seen lying between the kidney and the aorta, practically filling this region. The substance of the gland is cut up irregularly by venous sinusoids which form a network throughout the entire gland. The cortical cells are arranged in irregular columns which pass around these blood vessels and seem to form the foundation for all other elements

of the gland. The chromaffin cells have no regular arrangement, but are found in groups varying from two or three, up to thirty or forty cells each. The only regularity to be seen in the chromaffin groups is in their relation to the venous blood vessels. Except in exceptional cases, at least a part of each group is in direct contact with at least one of these blood vessels.

The connective tissue, which was first seen forming around the glands at the 168th hour of incubation, develops very slowly, but after about seventeen days of incubation a dense capsule has been formed around each gland. The connective tissue is confined almost entirely to the surface of the gland but in several places rather large masses of it may be seen entering the substance of the gland. This connective tissue breaks up at once and within the gland only very small fibers are found. These fibers are found only between the cords of cortical cells.

#### *The development of the venous system*

Owing to the structure of the adrenal glands of birds, the development of the blood vessels cannot be taken up separately for the cortical and chromaffin parts, as has been done for mammals.

The blood vessels of the adrenal gland develop so slowly that for specimens taken twelve hours apart, very little difference can be seen. For this reason it is very difficult to determine at exactly what age they first appear. The process is a gradual one and each condition blends perfectly into those immediately preceding, and those immediately following it.

As early as the 120-hour stage of development a few scattered blood cells are found throughout the anlagen of the glands, but no more are found than are present in the surrounding mesenchyme tissue. No direct connection with any blood vessels can be seen at this stage of development but in several places the wall of the subcardinal vein pushes out dorsally into the anlagen of the gland for a very short distance. No break or division of the wall of the vein can be seen at this time. Figure 6 shows the

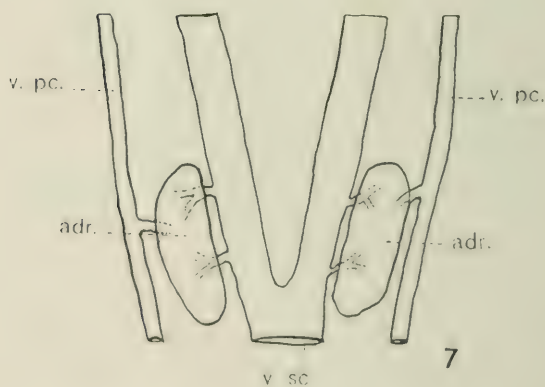


Fig. 6 Reconstruction of the vascular system in the adrenal region of a 120-hour chick embryo; dorsal aspect; *ad.*, anlagen of the cortical substance; *ao.*, aorta; *v. a.*, adrenal vein; *v. pc.*, postcardinal vein; *v. sc.*, subcardinal vein.

Fig. 7 Semi-diagrammatic drawing of the connection between the subcardinal and the postcardinal veins through the adrenal in a 168-hour chick embryo; ventral aspect; *adr.*, adrenal gland; *v. pc.*, postcardinal vein; *v. sc.*, subcardinal vein.

relation of the subcardinal veins to the glands and in several places the outpushing of the veins into the glands may be seen.

Sections of the glands at the 130-hour stage of development show that the subcardinal veins have pushed further into the



gland tissue than in the previous stage. No great modification of the gland can be seen and there is no apparent increase in the number of blood cells found in the gland. The evaginations of the subcardinal veins are very small at this time, but can be traced by the structure of their walls, which at this time are composed of only a single layer of endothelial cells. Their walls are of the same structure as those of the subcardinal veins at this period of development.

This development continues during the following fourteen hours so that at this period, 144 hours' incubation, these newly formed blood vessels have reached almost to the dorsal side of the gland. In several places, lateral branches are forming but these extend for only a very short distance. In no case was less than two of these venous trees found and in most cases three or four of them were present. This means that the venous blood vessels of the gland are formed, not by the division of one larger vein, but from several which push in from the subcardinal veins.

The development of the venous system continues by the branching of the vessels present in the gland until, after 168 hours of incubation, the gland has the appearance in section of having many irregular pieces cut out of its interior. At this period of development a new venous connection appears in the glands of birds (fig. 7). By the growth of the glands they come to lie ventro-median to the postcardinal veins. At this time, these veins turn ventrally to form the renal portal system, and in the region of the adrenals a branch is given off to the glands. Each postcardinal gives off a branch to the gland on its side of the body cavity. These veins push into the glands but instead of branching after the manner of the subcardinals and forming a system within the glands, they open directly into the blood vessels already present in the glands. In other words, they open into the venous tree already formed from the subcardinal veins. This condition naturally leads to the conclusion that there is a portal system formed in the adrenal glands of birds which might be called the adrenal portal system.

At this time the aorta and the postcardinal and subcardinal veins show connective tissue in their walls, upon which the

endothelial lining rests. This is not true of the venous blood vessels in the glands. These show no connective tissue in their walls and are larger than any capillaries found at this time. Owing to the difference in size and structure of these vessels I shall not call them capillaries, but shall adopt the term 'sinusoids,' proposed by Minot for this type of blood vessel.

The development of the sinusoids goes on steadily, but after 216 hours of incubation there does not appear to have been any appreciable increase in their number. The most noticeable change in the appearance of the gland is in the great increase in the size of the sinusoids. This growth, however, has in no way affected the nature of their walls, and they are still made up of a single layer of endothelial cells (fig. 5, *sl.*). The adrenal portal system has broken down at this time. It is a transitory condition, persisting through the eighth and ninth days of incubation only. It is significant that this connection with the post-cardinal vein should disappear as soon as the sinusoids have increased greatly in size. It should also be remembered that the greatest activity in the development of the chromaffin substance took place during the existence of this system.

Until after 240 hours of incubation, the sinusoids are found to open into the subcardinal veins in many places and by means of no very definite vessels. At this stage, 240 hours, the sinusoids seem to join into two groups near the posterior end of each gland and enter the subcardinal veins by means of four well defined veins, two for each gland. There is no evidence of a central vein running longitudinally through the gland, but this is to be expected since the sinusoids do not arise from the branching of a single vein, but from four or five veins which push in from the subcardinal vein. Each one of these veins then forms a system of sinusoids by repeated branching in the gland. This later development is then the combination of several of these systems at their bases to form a larger system. Since there are two veins found opening into the subcardinal vein, it follows that these numerous systems have combined to form two distinct systems of sinusoids in each gland. These systems are distinct only in point of origin, since there are numerous anasto-

moses formed between the sinusoids of the different systems and between the sinusoids of the same system.

The later development of the venous system is in no way remarkable. The terminal sinusoids continue to branch slowly up to the end of the period of incubation. At this period the gland is filled with sinusoids, so much so that in section they appear to occupy nearly one-half of the entire volume of the gland.

There can be no doubt as to the origin of the venous system of the adrenal glands of birds. It develops from inpushings of the subcardinal veins and penetrates the glands by means of numerous branches which in almost every case are directly in contact with the chromaffin substance.

#### *The development of the arterial system*

Observations on the development of the arteries of the adrenal glands reveal no conditions which are in any way out of the ordinary, and the condition is the same as that of any organ of this type.

The earliest connection found between the glands and any of the nearby arteries appears after 120 hours of incubation. At this time a small blood vessel is seen passing into the cortical substance of each gland from the anterior pair of mesonephric arteries. At this period the walls of these vessels are not very distinct, and within the gland no capillaries can be seen. For some time very little progress can be seen in the development of the arteries. After 144 hours of incubation the only increase in the complexity of the arterial system is the appearance of a very few indistinct capillaries within the cortical substance.

During the next twenty-four hours the arterial system of the glands develops rapidly and several new vessels appear during this period. An artery is given off by each of the anterior mesonephric arteries just before they enter the mesonephric glands. These arteries run anteriorly, one along the lateral border of each adrenal gland and two branches are given off to each gland by its respective artery, one near the posterior and the other near the anterior end of the gland, and each sends



branches into the cortical part of the gland. This does not occur, however, until after several branches have been formed on the surface of the gland and instead of a large artery penetrating the gland, directly, it is broken up and the branches are very small when they enter the substance of the gland. Still another arterial connection appears at this time. This is a small artery which runs directly from the aorta to the posterior

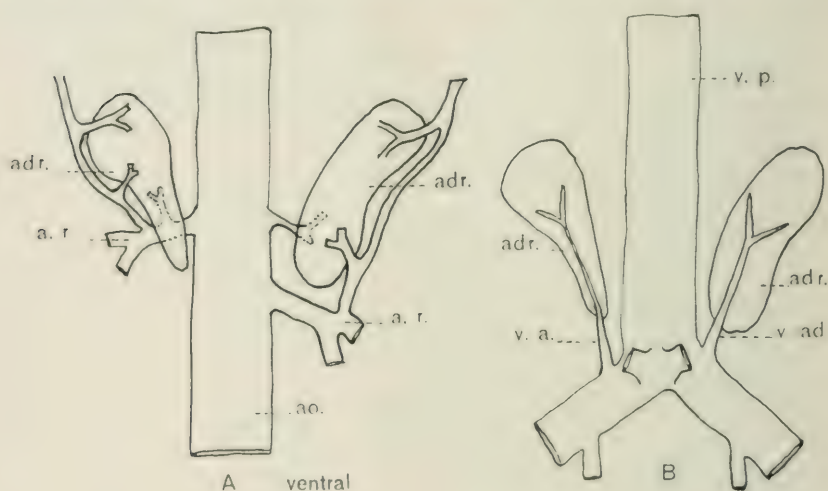


Fig. 8 A, The arteries of the adult fowl in the region of the adrenal glands: ventral aspect; *adr.*, adrenal gland; *ao.*, aorta; *a. r.*, renal artery. B, The veins of the adult fowl in the region of the adrenal gland; ventral aspect; *adr.*, adrenal gland; *v. a.*, adrenal vein; *v. p.*, postcava.  $\times 2$ .

end of the left gland. This artery also branches on the surface of the gland before entering the cortical substance. Within the glands all these arteries divide still further and the terminal branches are so small that in section they appear to be smaller than the blood cells. It is probable that in the living gland these capillaries are somewhat larger than in the sections observed. None of the capillaries within the gland were found in the chromaffin substance but there can be no doubt that at least the terminal branches sometimes penetrate this part of the gland.

Here again, the greatest growth of the chromaffin substance is accompanied by a correspondingly rapid development of the vascular system.

This practically completes the development of the arterial system of the glands. At a later period, about 192 hours, the connection between the mesonephric artery and the left adrenal disappears. This artery which disappears is not the one which arises from the branch of the mesonephric artery which runs anteriorly along the lateral edge of the gland. This artery runs directly from the mesonephric artery to the gland. The corresponding artery to the right gland remains (fig. 8). Aside from this modification there is no further change in the external arrangement of the arteries. Within the glands there is a slight increase in the number of capillaries and the nature of their walls undergoes a marked change. When first formed, the walls of the capillaries contain no connective tissue, but as the period of incubation draws to a close, a small amount of it may be seen in the walls of the larger ones. If any connective tissue is present in the walls of the smaller branches, the amount is so small that it cannot be seen in sections prepared by any of the ordinary methods.

#### *The glands of the adult bird*

In the adult bird, the adrenal glands lie just anterior to the bifurcation of the postcava, one on each side of the median line. They are about 1.5 cm. long and 0.5 cm. wide at the widest part. The right gland is roughly triangular, while the left is oval in outline (fig. 8, *ad.*). The internal arrangement of the cortical and chromaffin substances shows no change from the condition found in the embryo at the close of the period of incubation. In the natural increase in size of the glands it is the cortical substance, chiefly, which has increased in mass so that there is much more of this in proportion to the chromaffin substance than was present in the embryo. The same relation between the blood vessels and the tissue of the gland is found here as was described in the well advanced embryos. The sinusoids pass between the groups of chromaffin cells, while the capillaries lie in the cortical substance but each encroaches to a certain extent upon the territory of the other.

The trunks of the venous trees have increased greatly in size and form large central vessels which may be compared to the central vein of the adrenal gland of mammals. The sinusoidal character of the venous blood vessels persists in the adult and even in the largest vessels very little connective tissue is found in the walls. Near the close of the period of incubation, the venous blood was found entering the postcava by means of two separate veins from each gland. This condition is not found in the adult. The two veins anastomose on the ventral surface of the gland and the blood from both venous trees enters the postcava through a common vein (fig. 8).

The arterial system of the glands is essentially the same as that described for the embryo. Since this is true, it is evident that the mesonephric arteries from which they derived part of their blood supply have persisted as the renal arteries.

In the adult, the blood enters the gland by means of several arteries (fig. 8). It may enter the left gland directly from the aorta or through the anterior or posterior branches of that artery which arises from the renal artery and runs along the lateral border of the gland. The supply of the right gland differs from that of the left in that there is no direct connection with the aorta and in that there is a direct connection with the renal artery. The blood leaves both glands in the same way, entering the postcava at the bifurcation by means of a single vein from each gland.

#### SUMMARY

1. The anlagen which give rise to the cortical substance of the adrenal glands of birds appear as groups of cells which migrate dorsally from the peritoneal epithelium.

2. The chromaffin substance is derived from indifferent cells which wander in from the anlagen of the prevertebral sympathetic plexuses.

3. The chromaffin substance of the glands lies in contact with the venous blood vessels. The vessels of the arterial system are found almost entirely in the cortical substance. In general, this is the same condition as was found by Flint in the adrenal glands of mammals.



4. The entire venous system is derived from the subcardinal veins. Within the glands, the vessels of this system are sinusoidal in character.

5. During the period that there is the greatest influx of cells from the anlagen of the prevertebral sympathetic plexuses there is also the greatest activity in the development of the vascular systems. Is it possible that the relationship of cause and effect exists between the simultaneous activity in the development of these distinct systems of the adrenal glands.

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## MAST CELLS IN THE MENINGES OF NECTURUS, EASILY MISTAKEN FOR NERVE CELLS

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TWO FIGURES

In the study of the nervus terminalis of *Necturus maculosus*, attention has already been called (McKibben '11) to mast cells, the "clasmatoocytes of Ranvier," as they exist in the nasal region and in the meninges of this amphibian. The purpose of the present paper is not to attempt a description of these cells but rather to call attention to the fact that they may be easily mistaken for nerve cells when treated by some histological and neurological methods.

As is indicated in figure 1, these mast cells occur in great numbers in the dura mater. They are found also in the other meninges, along the olfactory nerve and about the nasal sac, as well as in the mesenteries and in the subcutaneous tissue where they were first described.

The cells in question (fig. 2) are elongated, irregular cells, usually with several long cytoplasmic processes. The nuclei of these cells seem poor in chromatin, taking a very feeble stain with basic dyes; but the cytoplasm surrounding the nuclei and that forming the long branching processes is seen to contain sharp granules which exhibit metachromatism. These cells, the "clasmatoocytes of Ranvier" (Ranvier '90, '93, '00) as described in *Amphibia*, have been shown by Jolly ('00) and by Maximow ('02, '06) to be identical with mast cells although of peculiar form. In *Mammalia*, where no similarity in form between the mast cells of Ehrlich and the clasmatoocytes exists, the confusion is impossible.



In figure 1, a drawing made from a whole mount of the dura mater of *Necturus*, the form, frequency and extent of the mast cells are shown. Their similarity to certain sympathetic nerve cells, in shape, size and extent, might lead one into serious error. That these are not nerve cells has been demonstrated thus: first,

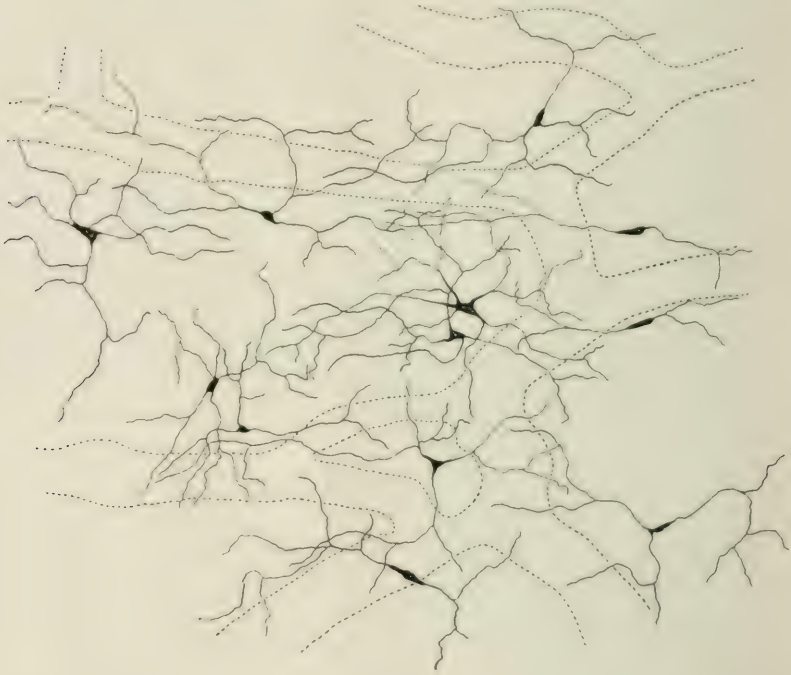


Fig. 1 Drawing, made with camera lucida, of the dura mater taken from the roof of the cranial cavity of *Necturus*; a whole mount of the dura mater fixed in formaline-Zenker's fluid and stained with Wright's stain. The blood capillaries are indicated by the dotted lines.  $\times 70$ .

the cytoplasmic granules have a different form and arrangement from that exhibited by the Nissl granules of nerve cells; second, they will stain intra-vitam with methylene blue and when so stained show the characteristic metachromatic tint; third, when treated with sulphuric acid and aqueous hematoxylin these granules fail to show the presence of iron which is characteristic of the Nissl granules of nerve cells. In these tests for iron, control

sections, known to contain nerve cells with Nissl granules, received exactly the same treatment as the sections containing the mast cells; so that the failure of the granules of the mast cells to react for iron was due, probably not to faulty technique, but to their chemical composition, when the Nissl granules in the same experiment gave the typical reaction.

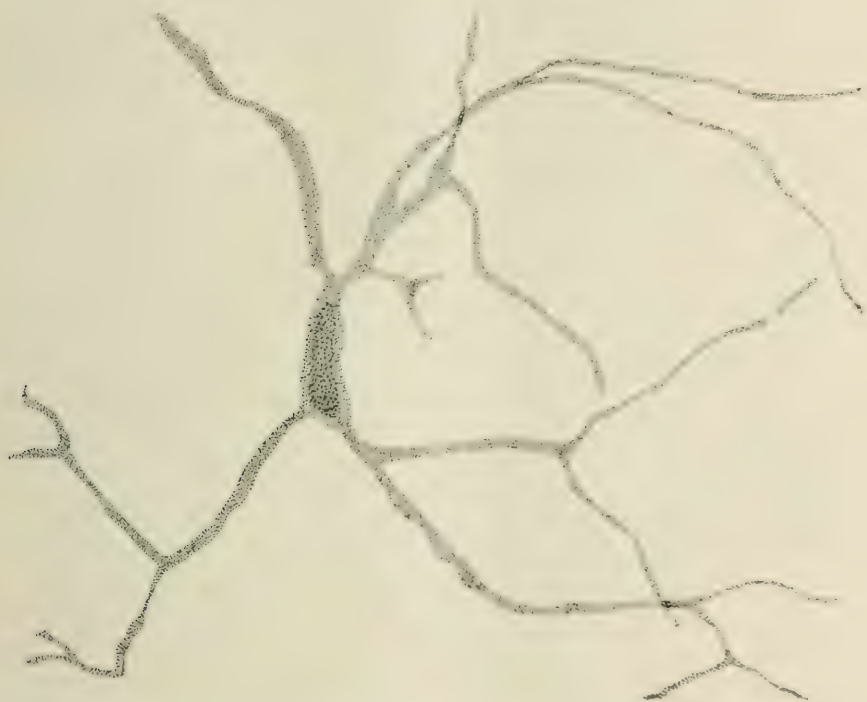


Fig. 2 Drawing, made with camera lucida, of a single mast cell in the dura mater from the floor of the cranial cavity of *Necturus*; a whole mount of the dura mater fixed in Formaline-Zenker's Fluid and stained with toluidine blue.  $\times 450$ .

Under certain conditions and in certain tissues, treatment of these mast cells in amphibia by the Golgi impregnation method, by the Cajal silver method and its modifications and by other methods, gives a picture in which it is well nigh impossible to determine whether one is dealing with sympathetic nerve cells or with these branched mast cells. Consequently in a study

in amphibia of certain tissues where sympathetic nerve cells and these mast cells may occur simultaneously, when methods are used by which it is impossible to differentiate between the two types of cells, the value of the observations is open to question. In Mammalia a similar confusion of nerve cells and certain cells of connective tissue is not altogether impossible.

The author wishes here to acknowledge his indebtedness to Professor R. R. Bensley and to the Anatomical Laboratory of the University of Chicago for assistance in this and other work.

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# A RACIAL PECULIARITY IN THE POLE OF THE TEMPORAL LOBE OF THE NEGRO BRAIN

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NINETEEN FIGURES (THREE PLATES)

While at the University of Michigan in 1906, I examined some casts of the interior of skulls which I had made in the Anatomical Laboratory of the Johns Hopkins University. My attention was struck by the apparently small size of the pole of the temporal lobe of the negro brain as compared with that of the white; and, proceeding to apply the calipers, the difference was demonstrated to be a measurable quantity. I then measured some brains from whites in the Anatomical Laboratory of the University of Michigan and others from both whites and negroes in the Anatomical Laboratory of the Johns Hopkins University, and at The Wistar Institute. I wish to thank Dr. McMurrich, Dr. Mall and Dr. Greenman for permission to use the material in their charge and for assistance in the work of making measurements.

## MATERIAL AND METHODS

The material studied consisted of 127 brains of negro males, 53 of negro females and 53 of white males (no white females) and measurements were made on each temporal lobe in two planes. The two planes selected are called basal and pole. For the basal plane I selected a plane passing horizontally through the temporal lobe, beginning posteriorly on the inferior surface at a point where a shallow depression is found in the inferior lateral border of the lobe by the upward projection of the petrous portion of the temporal bone, and ending anteriorly at a line where, if the plane were extended, it would leave the temporal

lobe to pass along the lowest part of the orbital surface of the frontal lobe. A straight-edged ruler laid alongside the brain will indicate the plane, if the edge of the ruler is on a level with the lower border of the frontal lobe and the depression in the temporal lobe made by the petrous bone.

The other or pole plane selected was parallel to the first and through a point 5 mm. vertically above the lowest projecting point of the temporal lobe. The two planes were measured both in their antero-posterior and transverse diameters.

The planes selected are not always the same in the brains examined, but they are the best that could be located and the results bear out the evidences of inspection of brains, and of skull casts, as well as of photographs of these and the evidences of Hrdlička's measurements of the skull. Therefore they are dependable if only as an approximation of the condition. The measurements cover only a small part of the temporal lobe, and that the mere extremity.

It may be added that in calculating the standard deviation, skewness of the curve and probable errors, Pearson's methods or Davenport's formulae have been followed. The median has been used instead of the mean because of the small number of individual measurements, the ease of calculation and the almost exact identity of the two.

#### MEASUREMENTS IN THE BASAL PLANE

The antero-posterior diameter of the basal plane shows the white males congregated about 52 to 56 mm., the negro males about 50 to 54 mm., and the negro females about 48 to 52 mm. The mean, that is to say the point midway between the most extreme cases, is 54 mm. for the whites, 52 mm. for the negro males and 48 mm. for the negro females. The median, that is to say the point with an equal number of cases on either side, is 54.3 mm. for the white males, 51.5 mm. for the negro males and 49.25 mm. for the negro females, while the extremes are separated by 26 mm. in the white males and in the negro males, but by only 22 mm. in the negro females. The averages of the antero-posterior diameter of the basal plane of the three

groups are 54.7 mm. for the white males, 51.8 mm. for the negro males and 49.4 mm. for the negro females and the mode is 55 mm. for the white males, 52 mm. for the negro males and 50 mm. for the negro females.

From these figures it will be seen that the antero-posterior diameter of the basal plane of the temporal lobe is greater in the white males than in the negro males and greater in the negro males than in the negro females, in the mean, on the average and by the median and the mode, the difference being about 3 mm. between the white males and the negro males and about 2 mm. between the negro males and females.

Hrdlička has measured the antero-posterior diameters of the fossae of the skull in various races, both in adult individuals and young, and in monkeys and other animals, and has determined that the temporal or middle fossa of the negro skull is absolutely shorter than that of the white or that of the Indian, and that it is also shorter in proportion to the total external length of the skull, this being especially noticeable in dolichocephals. His results are based on measurements of 55 negro skulls, male and female, compared with those of 90 white skulls, male and female, and those of the skulls of 20 Indian males. Although the points and planes selected are not exactly the same as those I used, yet there is no very great difference between his and mine, and the results of the two sets of measurements, the one on the skull and the other on the brain, are corroborative.

As regards the transverse diameter of the basal plane, the white males are grouped around 48 to 50 mm., the black males around 42 to 46 and the black females around 44 to 46. The mean is 49 mm. for the white male, 42 mm. for the negro male and 44 mm. for the negro female, and the median is 49 for the white male and 44 for both the male and female negro. The extremes are separated by 14 mm. in the white male, by 22 mm. in the black male and by 18 mm. in the black female. The averages are 49.3 mm. for the white male, 44.4 mm. for the negro male and 44.5 mm. for the negro female; and the mode is 50 mm. for the white male, 46 mm. for the negro male and 44 mm. for the negro female.



The transverse diameter of the temporal lobe in the basal plane is accordingly greater in the white male than in the negro male by about 5 mm. and it is about the same size in the two sexes of the negro, in the mean and in the average, by the median and by the mode. A comparison will show that there is a greater racial difference between the two sets of males in this, the transverse, diameter of the basal plane than in the antero-posterior one. Taking the averages, the difference between the two diameters in the white male brain is 54.7 minus 49.3 equals 5.4 mm., while that in the negro male brain is 51.8 minus 44.4 equals 7.4 mm., the transverse diameter of the basal plane as compared with the antero-posterior diameter being thus 2 mm. less in the negro male than in the white male, or, conversely, the antero-posterior diameter relatively to the transverse is 2 mm. greater in the negro male than in the white male. This difference may, perhaps, be better expressed by representing the relation by an antero-posterior transverse index in which the transverse diameter is taken as 100. Then the index for the male white is 110.9, while that for the male negro is 116.4. The difference between the averages of the diameters in the negro female is 4.9 mm., differing from that of the white male by 0.5 mm., while the index is 111, almost identical with that of the white male.

Hrdlička has measured the pituitary fossa in negro and white skulls, but his measurements do not extend to the body of the sphenoid bone and hence cannot be used for comparison.

#### MEASUREMENTS OF THE POLE PLANE

The measurements made at 5 mm. from the lowest projecting point of the temporal lobe are necessarily less accurate than those of the other two planes on account of the greater difficulty of obtaining the exact location of the plane, the variable shape of the temporal pole, etc. There is, however, an appreciable racial difference. The modes of the white males are about 26 mm. and about 34 mm.; those of the black males about 22 mm. and about 28 mm.; and those of the black females about 16 mm., about 20 mm. and about 26 mm. This multiple grouping is

due to the different shapes of the temporal pole, some being long, others round, while others are oval or oblong. These various shapes occur in each race-sex group and hence do not interfere with a fair comparison.

The transverse diameter of the pole plane is more homogeneous than the antero-posterior. The white males are grouped about 24 mm. and the negro males and females about 18 mm. The numbers of about the same value are more extensive than in the other arrays, indicating a tendency to a large grouping about the mode. A curve to illustrate this would be platycurtic, or flat-topped (McDonnell). The transverse diameter of the pole plane passes below the hippocampus.

#### THE SIZE OF THE TEMPORAL LOBE RELATIVE TO THE BRAIN WEIGHT AND SIZE

A comparison of the size of the temporal lobes with the total brain weights was made in the brains of 34 negro males, 21 negro females and 13 white males, and the results showed that there was a slight increase in the size of the temporal lobe with increase of brain weight, and that this increase was greatest in the white males and greater in the negro female than in the male. The brain weight of the white is greater than that of the negro and this might possibly account for the difference in the size of the temporal lobes as already determined. But this indicates that the lobes of the white are larger absolutely and relatively to brain weight, than those of the negro.

The same result is obtained by a comparison of the diameters of the temporal lobes with the diameters of the cerebral hemispheres to which they belong, length with length and breadth with breadth.

It will be seen that both in series and by averages the white has the advantage of the negro, the dimensions of the temporal lobes are actually greater in the white except in the case of the antero-posterior diameter of the basal plane, where the negro female has an advantage of 1 mm. in the average over the white male and of 2.6 mm. over the negro male.

## CONCLUSIONS

The general conclusions may be stated concisely as follows:

1. The size of the pole of the temporal lobe is less in the negro than in the white, and less in the negro female than in the male.
2. The differences are more pronounced in measurements taken below the hippocampus than in those which pass through that structure. Hence it is probable that
3. The hippocampus is larger in the negro than in the white and larger in the negro female than in the male.
4. The shape of the pole of the temporal lobe is different in the two races, being slightly more slender in the negro, and almost the same size in the two races antero-posteriorly
5. The differences are not only absolute but are also relative to the weight and size of the entire cerebral hemispheres.

The brains collected at Tulane University confirm the evidence in relation to the temporal lobe of the brains examined at the University of Michigan, at The Wistar Institute, and at the Johns Hopkins University. The brains examined at Tulane University were preserved in a uniform manner, but the brains examined in the other places were not, and the differences noted at Tulane University are more distinct than elsewhere.

## NOTE CONCERNING RECENT OBSERVATIONS ON THE NEGRO BRAIN

The brains examined here were preserved in the following manner: The bodies from which the brains were removed were injected with the usual Souchon solution as soon after death as possible, usually at least twenty-four hours after, and they were allowed to remain another twenty-four hours before the brains were removed. The skull caps were sawed as low down over the forehead and occipital region as practicable to remove the cap without disturbing the brain, and after the removal of the brain it was weighed and placed in 10 per cent formalin solution, base up, fitting it into the skull cap. The brains were found to harden readily, and to retain their shape especially well in the region of the temporal lobes. The skull cap being the shape of the vertex this also retains its shape. Should the brain be soft it may spread a little over the cut sides of the skull cap,



but if the brains are fitted well into the skull cap this seldom occurs.

In a test of the accuracy of my powers of observation, nine brains were selected at random without knowing their race character, and from the temporal lobes alone I judged the race correctly in all except two, which I called white, whereas they were from light-skinned mulattoes.

The brains have been measured in various dimensions, and observations as to the size of the pons, cerebellum, convolutions, etc., have been made, but these are reserved for future publication.

The temporal lobe of the brain may be described better than it can be measured. The upper part of the lateral side of the lobe in the negro brain is flat and the lateral side is also flat as it turns downward, inward and forward straight to the tip or pole of the lobe. In the white brain the upper part of the lateral side of the lobe is round, and the lateral side is also round and instead of passing downward as a flat surface it makes a graceful rounded sweep inward to the pole of the lobe.

The medial surface of the temporal lobe is almost perpendicular in the white brain, but in the negro it slopes outward. This makes the temporal lobe of the white brain appear to turn inward at the pole, whereas in the negro brain it is directed downward.

The pole of the temporal lobe is more slender, smaller and narrower in the negro than in the white brain.

The temporal pole of the brain of the negro female is more like that of the white than is the brain of the negro male, especially on the lateral surface, and this is due to the rounded surface of the female negro brain and the angular surface of the male negro brain.

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# PLATE 1

## EXPLANATION OF FIGURES

1 Skull casts; side view. Note the narrow pole of the temporal lobe in the negro skull cast.

<i>White male</i>	<i>White male</i>	<i>White male</i>	<i>Negro male</i>	<i>Negro male</i>
1245	1245	1216	1582	1582
Dura	Dura	No dura	Dura	Dura

The numbers refer to the serial number of brains (subjects) at the Johns Hopkins Anatomical Laboratory.

2 Skull casts; view from below. Note the wide pole of the temporal lobe in the white skull cast; note also the great width of the space between the poles in the negro casts.

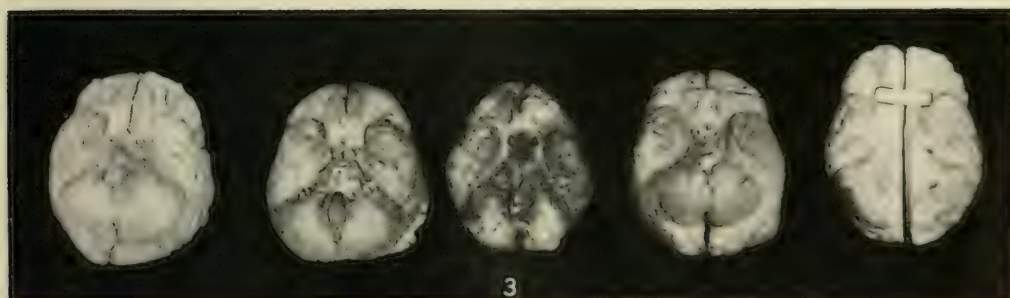
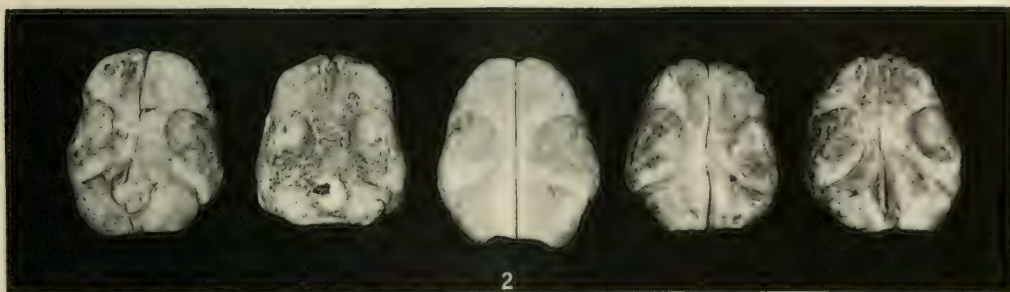
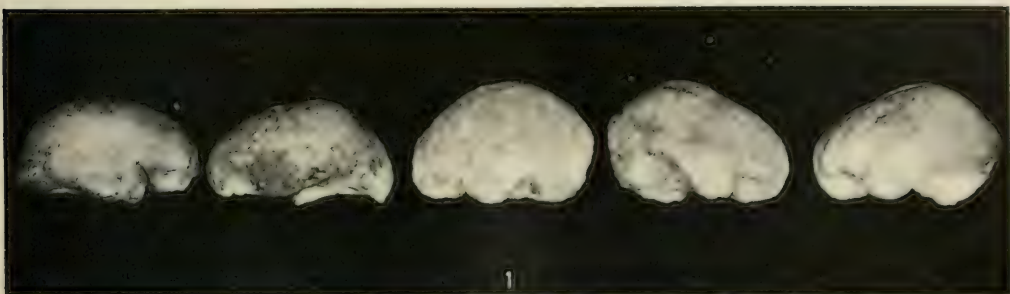
<i>Negro male</i>	<i>Negro male</i>	<i>White male</i>	<i>Negro male</i>	<i>Negro male</i>
1247	1330	1216	1212	1217
Dura	Dura	No dura	No dura	No dura

3 Brains; view from below. Note the narrow poles of the temporal lobes of the negro brain and cast and the wide space between them.

<i>White brain</i>	<i>White brain</i>	<i>Negro brain</i>	<i>White brain</i>	<i>Skull cast</i>
Ann Arbor	Ann Arbor	Ann Arbor	Ann Arbor	Negro male
				1212
				No dura

4 Skull casts; front view. Note the narrow temporal poles and the wide space between them in the negro skull casts.

<i>Negro male</i>	<i>Negro male</i>	<i>White male</i>	<i>Negro male</i>	<i>Negro male</i>
1219	1217	1216	1330	1247
Dura	No dura	No dura	Dura	Dura





## PLATE 2

### EXPLANATION OF FIGURES

The outlines in figures 5 to 19 inclusive were made from projections through a lens with the brains each at the same focal distance from the lens. The gyri and sulci of the temporal lobes are given, as only these are in focus. The outlines show the temporal lobes as if viewed from above through transparent brain substance.

5 Brain 2; white male; age 25; cause of death, pulmonary tuberculosis. Total brain length, right hemisphere 16 cm.; left hemisphere 16 cm.; total brain breadth 14 cm.; total brain height 10.1 cm. weigh 1304 grams. Note the wide temporal lobes.

6 Brain 1; negro male, age 65; cause of death, nephritis. Total brain length, right hemisphere 17 cm.; left hemisphere 17.2 cm.; total brain breadth 14 cm. Weight 1361 grams. Note the narrow temporal lobes.

7 Brain 6; white male; age 55; cause of death, pulmonary tuberculosis. Total brain length, right hemisphere 16.2 cm.; left hemisphere 16.5 cm.; total brain breadth 14 cm.; total brain height 10.4 cm. Weight 1332 grams. Note the wide temporal lobes.

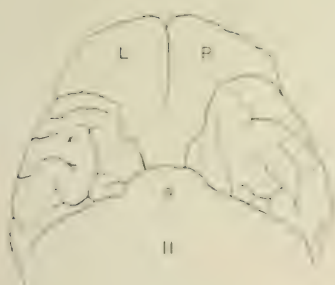
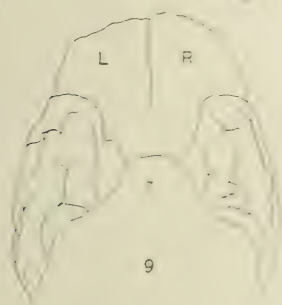
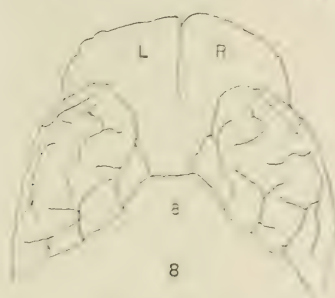
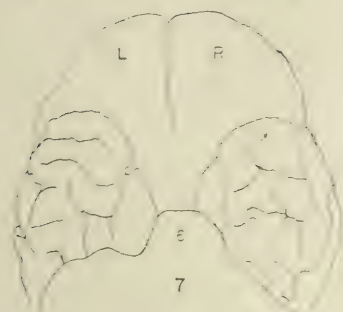
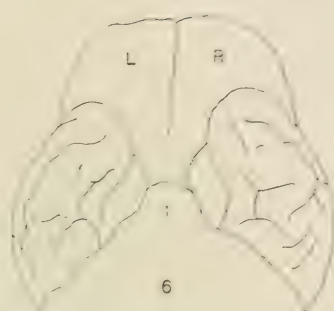
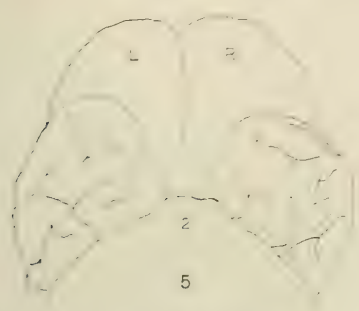
8 Brain 8; negro male; age 48; cause of death, tuberculosis. Total brain length, right hemisphere 16.7 cm.; left hemisphere 16.8 cm.; total brain breadth 13.6 cm. Weight 1503 grams. Note the narrow temporal lobes.

9 Brain 7; negro female; age 75; cause of death, unknown. Total brain length, right hemisphere 16.1 cm.; left hemisphere 16.1 cm.; total brain breadth 12.4 cm. Weight 992 grams. Note narrow tips of the temporal lobes.

10 Brain 10; white female; age 23; cause of death, lobar pneumonia. Total brain length, right hemisphere 15.5 cm.; left hemisphere 15.7 cm.; total brain breadth 12.6 cm.; total brain height 10.6 cm. Weight 1155 grams. Note the wide temporal lobes.

11 Brain 9; negro male; age 38; cause of death, pulmonary tuberculosis. Total brain length, right hemisphere 16.5 cm.; left hemisphere 16.4 cm.; total brain breadth 13.5 cm.; total brain height 10.6 cm. Weight 1304 grams. Note the narrow poles of the temporal lobes.

12 Brain 3; mulatto male; age 53; cause of death, nephritis. Total brain length, right hemisphere 16.8 cm.; left hemisphere 17 cm.; total brain breadth 13.6 cm. Weight 1389 grams. Note the wide temporal lobes like those of the white.



### PLATE 3

#### EXPLANATION OF FIGURES

13 Brain 14; negro male; age 38; cause of death, pulmonary tuberculosis. Total brain length; right hemisphere 15.8 cm.; left hemisphere 15.6 cm.; total brain breadth 12.1 cm.; total brain height 9.9 cm. Weight 1106 grams. Note the narrow temporal lobes.

14 Brain 4; negro male; age 65; cause of death, nephritis. Total brain length, right hemisphere 16.7 cm.; left hemisphere 16.7 cm.; total brain breadth 12.1 cm. Weight 1219 grams. Note the narrow temporal lobes.

15 Brain 12; negro female, age 70; cause of death, arterio-sclerosis. Total brain length, right hemisphere 16.3 cm.; left hemisphere 16.7 cm.; total brain breadth 12.3 cm.; total brain height 10.9 cm. Weight 1169 grams. Note the narrow points of the temporal lobes.

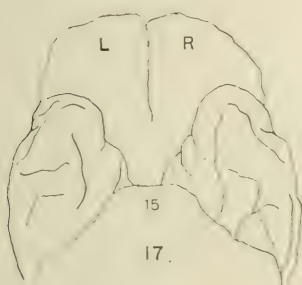
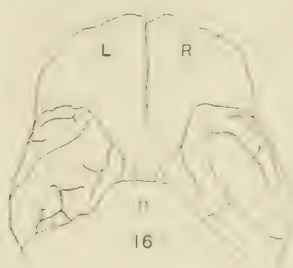
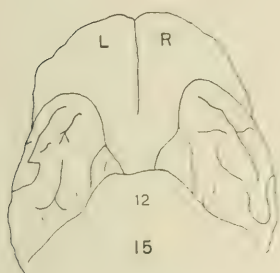
16 Brain 11; mulatto female; age 24; cause of death, pulmonary and intestinal tuberculosis. Total brain length, right hemisphere 17 cm. (distorted; left hemisphere 16.4 cm.; total brain breadth 12.8 cm. Weight 1155 grams. The temporal lobes are as wide as in the white.

17 Brain 15; negro male; age 28; cause of death, lobar pneumonia. Total brain length, right hemisphere 17.1 cm.; left hemisphere 17.1 cm.; total brain breadth 12.7 cm. Weight 1304 grams. Note the narrow point of the temporal lobes and the large hippocampus.

18 Brain 13; negro female; age 23; cause of death, pulmonary tuberculosis. Total brain length, right hemisphere 16.6 cm.; left hemisphere 16.4 cm.; total brain breadth 12.7 cm. Weight 1219 grams. Note the narrow point of the temporal lobes and the large hippocampus.

19 Brain 5; mulatto male; age 39, cause of death, lobar pneumonia. Total brain length, right hemisphere 16.8 cm.; left hemisphere 16.8 cm.; total brain breadth 12 cm. Weight 1233 grams. The temporal lobe is wide as in the white.







## AN ABNORMALITY IN THE INTESTINE OF *NECTURUS* *MACULOSUS* RAF.

LESLIE B. AREY

SIX FIGURES

Morphological abnormalities in *Necturus* have been described by many workers and, because of their frequency, have long since ceased to occasion astonishment. A great majority of reported cases, however, are based on skeletal variations, while variations in the soft parts either occur less frequently, or, what is more probable, become 'smoothed out' in subsequent development and thus escape attention. The following case, involving the fusion and communication of a loop of the ileum with the rectum, would seem worthy of mention if only on account of its novelty and its bizarre nature. Acknowledgment is due Dr. E. L. Mark for critical reading of the manuscript.

The specimen was a sexually mature female, the vascular system of which, fortunately, had been injected for study in comparative anatomy.

The rectum (fig. 1, *rt.*), which has the usual proportions, joins the cloaca in an essentially normal fashion. The ileum, traced toward the stomach, proceeds cranial from the point where it merges into the rectum 1.5 cm. and then turns sharply and runs caudad for 3.0 cm., fusing abnormally with the rectum about 1.5 cm. from the end of the latter.

This reflexed limb of the ileum (*il.rfx.*) is, for the most part, somewhat smaller than the normal ileum (*il.no.*), but its caudal third becomes enlarged to form a prominent swelling which, subterminally, joins the right lateral side of the rectum in a well defined junction. The rectum receives the normal ileum a little distance in front of the union just described, on the right ventro-lateral side of the rectum.



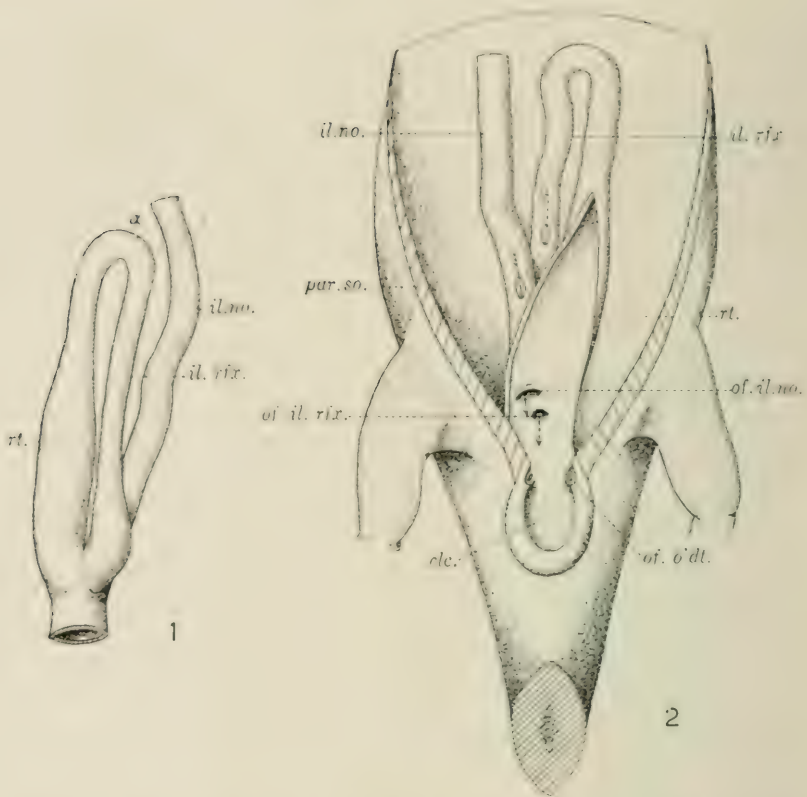


Fig. 1 Dorsal view, rectum severed just cranial to the oviducal apertures.  $\alpha$ , transitional region, where the dorsal mesentery transfers its primary connection from the normal ileum to the rectum; *il.no.*, normal ileum; *il.rfx.*, reflex ileum; *rt.*, rectum.

Fig. 2 Ventral view into the body cavity, with the rectum opened by a median ventral incision. Arrows with full and broken lines show the relation of the normal and reflex ileum to their respective rectal orifices. *clc.*, cloaca; *il.no.*, normal ileum; *il.rfx.*, reflex ileum; *of.il.no.*, orifice of normal ileum into rectum; *of.il.rfx.*, orifice of reflex ileum into rectum; *of.o'dt.*, oviducal orifice into cloaca; *par.so.*, cut edge of body wall; *rt.*, rectum.

That the ileo-rectal loop does not end blindly at the swollen region of union was first tested experimentally by forcing a colored liquid backward in the reflex portion of the ileum, whence it appeared in the cloaca; later this was substantiated by dissection.

When the cloaca and rectum were opened by a mid ventral incision (fig. 2), the orifices of the normal ileum and of the reflexed ileum into the rectum were easily demonstrable. The former enters by an aperture only slightly smaller than its lumen, and nothing that can be called a typical sphincter occurs, although sections examined under the microscope showed the circular muscles to be somewhat aggregated at this point; the latter, on the contrary, enters by an aperture of about the diameter of a pin, located at the bottom of a deep cup-shaped collar, a little dorsad and caudad to the entrance of the ileum proper. Sections

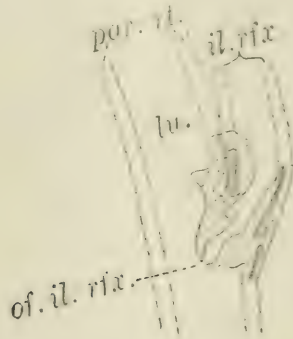


Fig. 3 Schematic longitudinal section through the region of union of the reflex ileum and the rectum, to show the relation of the section seen in figure 4. (full lines) to the adjacent parts (broken lines). *il. rfx.*, reflex ileum; *lu.*, lumen of rectum; *of. il. rfx.*, orifice of reflex ileum into rectum; *par. rt.*, wall of rectum.

of this region (fig. 4) show a conspicuous band of circular muscles surrounding the constricted opening; this presumably constitutes a sphincter. Normally no sphincter occurs at the junction of the ileum and rectum in *Necturus*.

The large, essentially normal sized aperture of the ileum proper is easy to understand from the standpoint of functional necessity, but the occasion of the establishment and perpetuation of a sphincter in an apparently useless loop is not so evident.

The whole ileo-rectal loop was found full of faeces—a pertinent fact.

The membranes supporting this region are not without interest. The rectum, and the ileum as far as the anterior bend of the ileo-rectal loop, are supported in the normal manner by the mesentery. Craniad to the loop the mesentery is attached to the ileum proper, while caudad to this transition point (fig. 1,  $\alpha$ ) the ileum is not primarily supported to the body wall. A narrow membranous sheet, however, (fig. 6, *ms' enr. i'il.*) connects the normal ileum with the reflex ileum; the reflex ileum and the ileo-rectum (fig. 6, *ms' enr. rt-il.*) likewise are similarly connected.

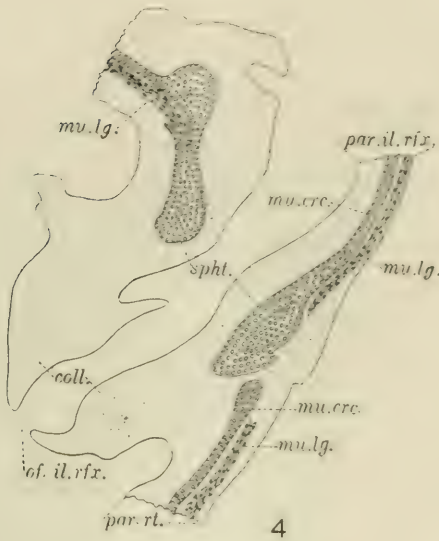
All these intestinal parts appeared to be in a well nourished condition. Blood vessels from the mesenteric vein and posterior mesenteric arteries follow their usual courses in the mesentery, and smaller branches ramify through the walls of both the normal and the reflex ileum.

The time of the establishment of this abnormality was, presumably, early in the development of the animal—an assumption to which the condition of the supporting membranes points. The narrow membranous sheets between the intestinal limbs are evidently continuations of the primary mesentery; this is well shown at the transition point (fig. 1,  $\alpha$ ) where the mesentery ceases to support the ileo-rectal loop and directly supports the normal ileum.

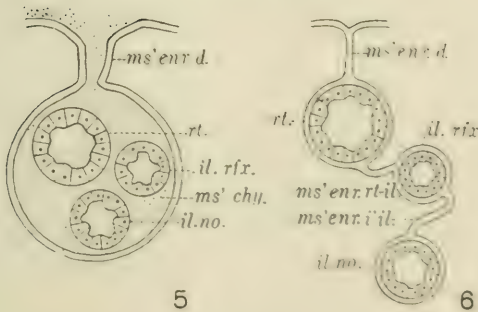
The origin of this condition can be explained by assuming (fig. 5) a loop of the embryonic intestine to have been reflexed *inside* the mesenteric fold which contained the gut loosely embedded in a mesenchymatous matrix. When, later, this common supporting fold became closely applied to the three intestinal members (fig. 6), the condition of the membranes, as described above, was effected. To complete the process, the caudal end of the loop had only to fuse with the rectum and to establish communicatory openings with it.

The finding of the ileo-rectal loop full of faecal matter stimulates speculation concerning the rôle which the loop played with regard to its contents. It is possible that material entering the rectum from the normal ileum merely backed up from the rectum into the loop until the latter became filled. If, however, the intestinal movements proceeded in the normal direction,





4



5

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Fig. 4 Longitudinal section through the region of the sphincter of the reflex ileum ( $\times 13$ ). *coll.*, collar extending into lumen of rectum; *mu.crc.*, circular muscles; *mu.lg.*, longitudinal muscles; *of.il.rfx.*, orifice of reflex ileum into rectum; *par.il.rfx.*, wall of reflex ileum; *par.rt.*, wall of rectum; *sph.*, sphincter.

Figs. 5 and 6 Diagrammatic cross sections to show how the embryonic ileum, if reflexed *inside* the mesenteric fold (fig. 5), could produce the conditions of the supporting membranes found in the adult specimen (fig. 5). *il.no.*, normal ileum; *il.rfx.*, reflex ileum; *ms'chy.*, mesenchyme; *ms' enr.d.*, dorsal mesentery; *ms' enr. i'il.*, interileal mesentery; *ms' enr. rt-il.*, recto-ileal mesentery; *rt.*, rectum.

such backing up could be effected only in opposition to peristalsis, which would tend continually to empty the loop of its contents. That a certain amount of substance might enter through the sphincter and proceed around the loop is possible, though hardly probable. A third possibility is that the reflex ileum had its peristaltic polarity reversed. Since the flexure probably occurred before the muscles were functional, this view is entirely tenable. Part of the faeces collected in the rectum, where peristalsis is poorly developed, would thus be captured by the abnormal ileal loop, would be passed in an abnormal direction through the loop and out through the sphincter; the normal activity of a sphincter would favor this view. That any of these hypothetical processes involved more than a small fraction of the total faecal matter is highly improbable, and speculation as to what might have happened can easily be carried too far.

It is interesting to note that here, in nature, we find the essential condition effected by the modern surgical operation, originated by Sir W. Arbuthnot Lane, whereby the ileum is connected to the rectum, and the colon is thus short circuited in the conduction of food.

# THE DEVELOPMENT OF THE HYPOPHYSIS OF AMIA CALVA

P. E. SMITH

*From the Hearst Anatomical Laboratory, University of California*

TEN FIGURES

## HYPOPHYSIS OF AMIA

The origin of the hypophysis in the various forms studied has been a source of disagreement, both in observation and in interpretation. The majority of those who have worked upon this question consider that the hypophysis arises from ectoderm; however, a few well known observers, Kupffer ('94), Valenti (in a series of papers), Nusbaum ('96), and Gregory ('02) state that it has also an entodermal contribution. The only author who describes the hypophysis as entirely entodermal in origin is Prather ('00) in *Amia*. That such an interesting phylogenetic anomaly is described seemed to the writer to warrant a further examination of this form, especially as Dean ('96) described this structure in this form, as of epiblastic origin.

The various specimens studied range in age from soon after the closure of the neural tube up to an 18 mm. stage. In fixation and staining especial care was taken to preserve the yolk granules and cell boundaries so that these most valuable structural features would be retained. The ages could be determined only by comparison with the excellent figures of Dean ('96).

Figure 1 is from a median sagittal section of an embryo surrounding about 225° of the yolk. It corresponds to the stage figured by Dean (fig. 2) and so is about 142 hours old, or the same age as the specimen figured by Prather (fig. 1). Beneath the hypothalamic region of the brain is the foregut and the stomo-



daeum separated by an imperfectly formed oral plate. The ectoderm forming the floor of the stomodaeum differs somewhat from that of the roof. That of the floor is two layers in thickness having a more acidophilic cuticular layer and a more basophilic basal layer. The ectoderm of the roof has an imperfectly formed cuticular layer, and a definite, cytoplasmic-rich basal layer, while between the two is an irregular single or double layer of cells rich in yolk and not easily distinguished from the entoderm cells at the juncture of the stomodaeum with the foregut.

A growth of this basal layer caudad extends from both the floor and the roof of the stomodaeum. That from the floor contributes to the dental ledges while that from the roof is more extensive and is the hypophysial rudiment. The cells composing this mass are more protoplasmic than the entoderm cells which have many large yolk granules. Their cell boundaries are also more distinct than those of the entoderm cells. There is no limiting membrane between the entoderm and this hypophysial rudiment, at this or later stages, but in specimens properly stained and differentiated the two varieties of cells can easily be distinguished from each other. A strand of cells connecting the hypophysis to the basal layer of the ectoderm is present. This connecting strand was evidently overlooked by Prather, for he describes and figures (fig. 3) at a little later stage (160 hours) and in a position farther caudad, a nest of cells of this character. His figure also strongly suggests that his hypophysial rudiment is

#### ABBREVIATIONS

<i>b.v.</i> , blood vessel	<i>l.t.</i> , lamina terminalis
<i>ect.</i> , ectoderm	<i>mes.</i> , mesenchyme
<i>ect.m.</i> , basal layer of ectoderm	<i>m.r.</i> , mamillary recess
<i>ent.</i> , entoderm	<i>op.c.</i> , optic commissure complex
<i>ent.d.</i> , entoderm, deep layer	<i>op.</i> , optic chiasma
<i>ent.s.</i> , entoderm, superficial layer	<i>pr.s.</i> , premandibular somite
<i>f.g.</i> , fore gut	<i>r.p.op.</i> , recessus post opticus
<i>hyp.</i> , hypophysis	<i>r.pr.op.</i> , recessus preopticus
<i>inf.</i> , infundibulum	<i>st.</i> , stomodaeum
<i>l.</i> , limit between ectoderm and entoderm	<i>s.v.</i> , saccus vasculosus
	<i>v.l.</i> , vestigial lumen

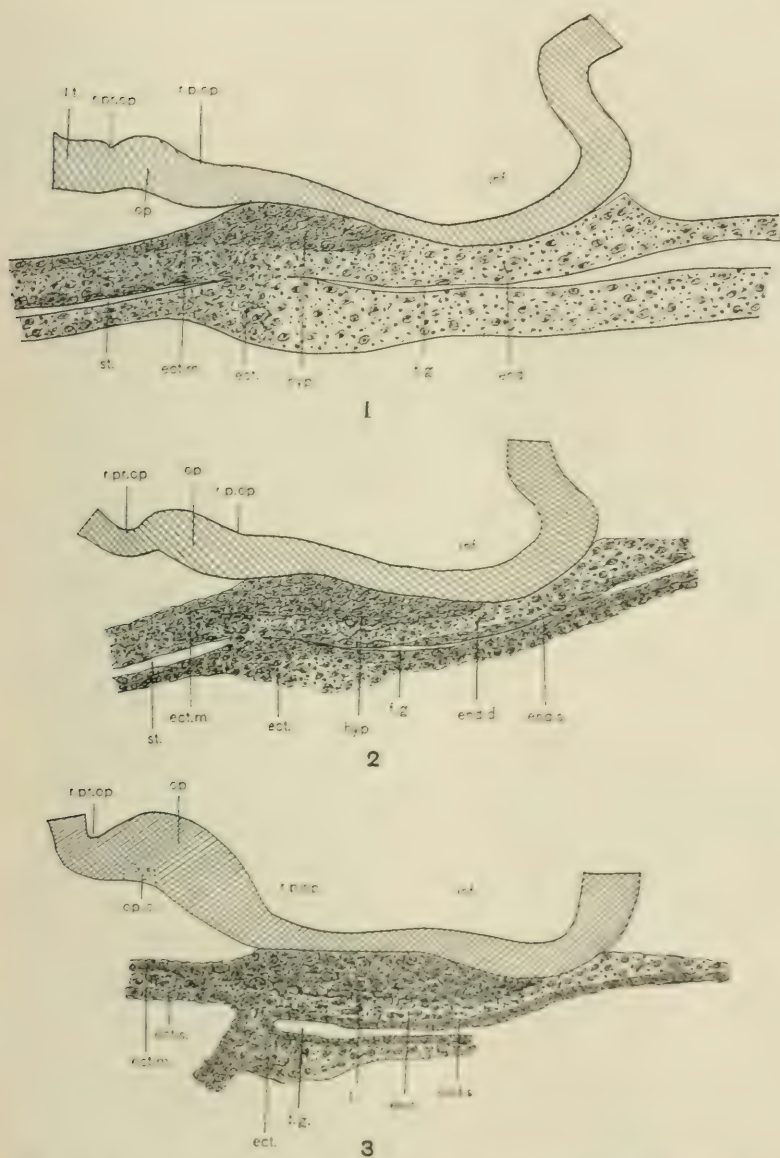


Fig. 1 A median sagittal section of a larva surrounding  $220^\circ$  of the yolk; estimated age, 142 hours.  $\times 200$ .

Fig. 2 A median sagittal section of an embryo surrounding  $290^\circ$  of the yolk; estimated age, 160 hours.  $\times 200$ .

Fig. 3 A median sagittal section of an embryo at the time of hatching.  $\times 200$ .

connected to the ectoderm. In cross-section this cell mass appears as a single ovoid nest composed of both flattened and oval shaped cells.

In a later stage (fig. 2) corresponding to Dean's figure 5, larva surrounding  $290^\circ$  of yolk, this cell mass can be still more easily distinguished from the entoderm. It has extended farther caudad and is separated from the cuticular layer of the entoderm by an irregular double layer of yolk laden cells. The connecting strand is still very evident.

Still older specimens (figs. 3, 4, 5) shortly after hatching, show that further growth of this structure has taken place but the relations remain unaltered. In figure 4 the deeper, yolk rich, layer of entoderm shows particularly well. Directly beneath the hypophysis, to an extent, but particularly at the sides the entodermal cells have become flattened, and to them especial attention will be called later. In figure 5, two sections cephalad to figure 4, these entodermal cells are still more flattened.

In a 6 mm. specimen (figs. 6, 7) the hypophysis has assumed nearly its adult position. In the dorsal and lateral portions the cells are either oval or round, and show a radial arrangement towards a minute cavity, the vestigial lumen. Ventral to the lumen the cells are few in number, flattened, and do not radiate towards the cavity. It is to the juncture of these flattened, ectodermal, hypophysial cells with the entoderm, that particular attention is directed. In the early stages of the hypophysis they were easily distinguished from the entoderm of the foregut. During development, however, the entoderm has been losing more and more of its yolk granules until its appearance is practically like that of the hypophysis, and so from this stage on these

Fig. 4 Cross-section through the cephalic portion of the hypophysis of an embryo at the time of hatching.  $\times 200$ .

Fig. 5 Same series as figure 4; two sections caudad.  $\times 200$ .

Fig. 6 A median sagittal section of a 6 mm. specimen.  $\times 200$ .

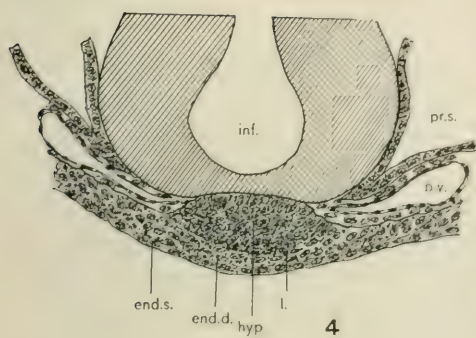
Fig. 7 Cross-section of a slightly older specimen.  $\times 200$ .

Fig. 8 A median sagittal section of an 8 mm. specimen.  $\times 200$ .

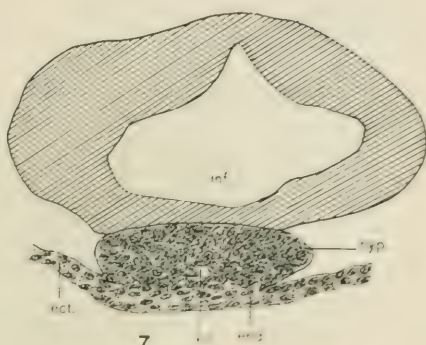
Fig. 9 A median sagittal section of an  $8\frac{1}{2}$  mm. specimen.  $\times 200$ .

Fig. 10 A median sagittal section of a 10 mm. specimen.  $\times 200$ .

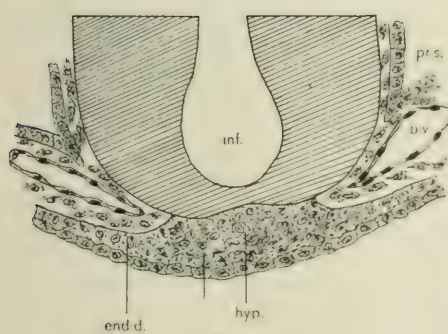




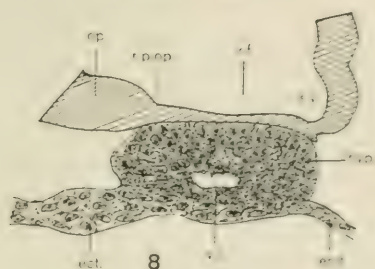
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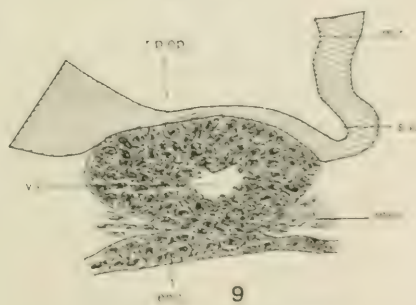
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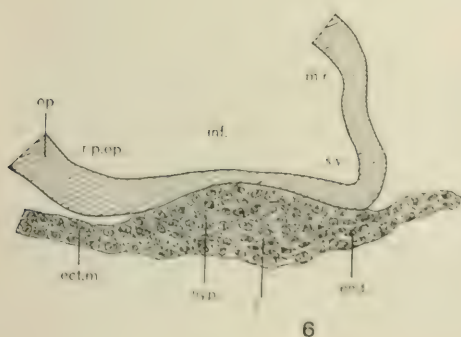
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entodermal cells must be identified largely by their position. In a cross-section of this age (fig. 7) the same condition is evident.

Later stages (fig. 8, 8 mm.; fig. 9, 8½ mm.) show the separation of the hypophysis from the pharynx. This separation takes place by or becomes apparent with a mesodermal ingrowth into the intercellular clefts at the sides and base of the hypophysis. Examination of sections at this critical stage shows convincingly that part of the flattened cells of the deeper layer of the entoderm become separated from the pharynx and enter into the formation of the hypophysis. Tracing these entodermal cells which border the ectodermal hypophysial rudiment, through the successive stages, has been the most interesting and difficult part of the study. At the time of the separation of the hypophysis from the mouth they can not be determined, structurally, from the cells of ectodermal origin. It is only by identifying them at the latest possible stage by their staining reaction and then by their position and relation that it can be said with considerable probability that they do enter into the formation of the adult hypophysis. The process is much like that described by Gregory ('02). In his figures (figs. 29, 30, 31) he indicates a flattening of the entodermal cells and their inclusion into the ectodermal hypophysial rudiment. However, he carries the process much further than here described and includes all the thickened entodermal mass caudad of the ectodermal hypophysial anlage in the formation of this structure. The difficulty of determining the limit between ectoderm and entoderm in *Amia* was also mentioned by Dean.

The vestigial lumen has been well described by Prather and needs little additional attention. A limiting membrane as definite as he indicated was not noted. Extending away from the lumen are many intercellular spaces. It appears as if by secretion the cells force themselves apart. This is even more apparent later with the increase in the lobulation of the gland and the communication of the cavities of the various lobes with each other by intercellular spaces.

## SUMMARY

The development of the hypophysis in the ganoids has been worked out with rather uncertain results. Haller ('98) has cast doubt upon the work of von. Kupffer; Balfour and Parker ('82) were in doubt as to their own results; while Dean differs from Prather in the work upon *Amia*. This confusion is partially due, perhaps, to the developing adhesive organ, but primarily to the difficulty of distinguishing between the ectoderm and the entoderm. Their union is intimate from the first appearance of the hypophysis, and it is only by noting from the first the caudal growth of the basal layer of the ectoderm to form the hypophysial rudiment, that the origin of this gland can be given. The process in *Amia* differs in no essentials from that in the other teleosts and the amphibia. The relation of the entoderm of the foregut to the gland is of interest in showing the plasticity of the tissues. This adaptability of tissue or germ layers is especially well exemplified in the formation of the hypophysis in this form where the union of two germ layers is particularly close. As observed by the author in some other forms as well, the contiguity of the entoderm to the gland leads to the modification of the former and a change from its normal function to that of another germ layer. Whether this is due to the influence of the nervous system, the inherent tendency of the tissue, or some other factor or factors, cannot be stated at this time.

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## A BRAIN MACROTOME

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### TWO FIGURES

In recent work on the asymmetry of the basal ganglia it became necessary to obtain sections of the brain of uniform thickness which were traced on wax plates of similar thickness. The sections were 3.5 mm. thick. In order to facilitate the work and render it accurate, a macrotome was devised, which has since been modified from suggestion by Dr. G. Y. Rusk of the Department of Pathology.

The base and standard of the instrument are cast separately and screwed together. The base is Y-shaped, similar to that of a compound microscope, measuring 29 x 19 cm. and 2 cm. thick, of cast-iron, affording a firm support to the instrument. The standard measures 2 x 5 cm. and 32 cm. long, and is provided at the back with two flanges for additional strength.

Near the top of the standard is fixed a U-shaped piece enclosing the movable stage. This U-shaped piece measures 23 x 23 cm. and is 1 cm. thick. On the upper surface of each arm are laid two strips of plate-glass, separated from each other by bits of steel from the blade of the knife which is used for cutting, and held to the arm by lead washers and round-headed screws. The movable stage runs in a vertical groove fastened separately to the standard, and measures 19 x 18 cm. and 1 cm. thick. When raised to the level of the U-shaped piece, it fits accurately within the arms. To prevent drainage of liquids into the groove a flange is placed near the edge. The stage is adjusted by a screw with a millimeter thread pressing up against the bottom of the stage and giving to it a play of 10 cm. A milled head operates the screw, and by it an adjustment to a fraction of a millimeter may be made.

The knife is made from a clock spring, 30 cm. long, ground to a razor edge, mounted in a hack-saw back. Both edges are ground to permit the knife to be used either erect or inverted. The interval between the stage and the base gives ample space for the back of the inverted knife, the stage not approaching nearer the base than 12 cm. To use the knife, the upper strips of plate glass are removed, the blade inserted, and the glass screwed into place again.

In using the macrotome the brain is placed on the movable stage and the level to be sectioned adjusted to the level of the lower glass

strips. The knife cuts with a back-and-forth motion. The section is removed and the stage readjusted by raising it the required height.

The advantages of the instrument are: (1) Simplicity; there are no parts but what may be constructed by an ordinary technician. (2) Accuracy; the knife-blade being held tightly between the plate-glass strips, and the stage supported firmly from beneath, there is insured an accuracy in the thickness of the sections which for microscopic work is all that can be desired. (3) Convenience; the instrument occupies about the same room as a compound microscope, and can be carried very conveniently from place to place in the laboratory. (4) Cheapness.



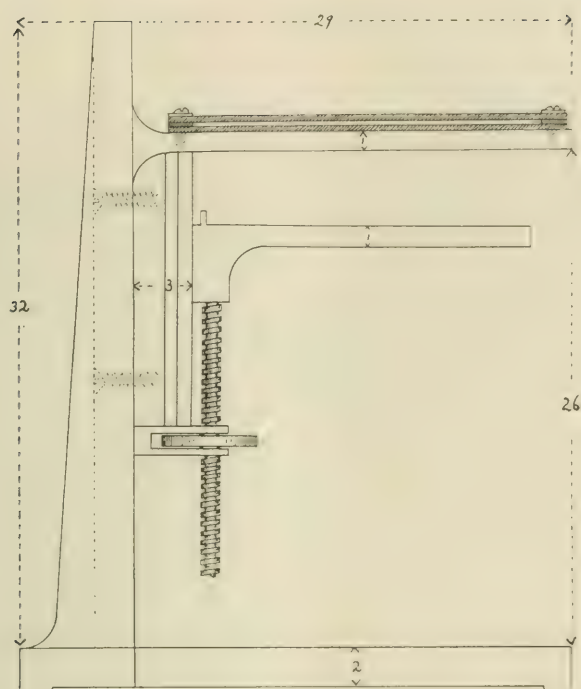


Fig. 1 Side view of macrotome; measurements in centimeters.

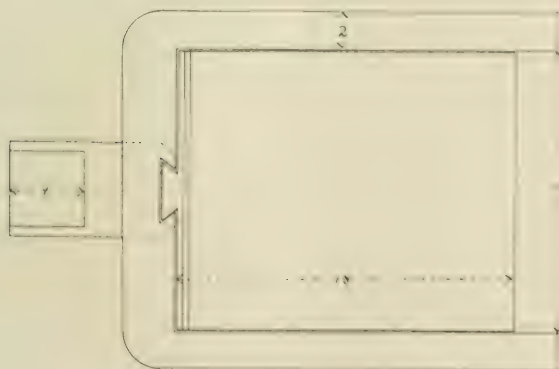


Fig. 2 Top view of macrotome; measurements in centimeters.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

**LABORATORY APPARATUS AND REAGENTS** selected for laboratories of chemistry and biology in their application to education, the industries, medicine and the public health including some equipment for metallurgy, mineralogy, the testing of materials, and optical projection. 580 pages, Philadelphia, 1914, Arthur H. Thomas Company. A very complete illustrated catalogue showing that great care has been exercised to give accurate descriptions of apparatus.

The Reagent list with analyses of the important makes is the only one of this kind published either in the United States or Europe, so far as we know, and it seems to us to afford the scientist a means of selecting his Reagents upon both the basis of purity and price, which has not been heretofore provided in any price list.

The following statement in the Preface to the volume is honest, clear, explicit and rather unique. "Our business is confined to the buying and selling of Apparatus and Reagents, mostly within the limits mentioned on the title page of this catalogue. We are not scientists, inventors or manufacturers and we are not equipped to design and experimentally develop scientific apparatus. We believe such work is properly done by the scientist in his laboratory, the manufacturer in his shop, or by the two in coöperation and that the function of the dealer advantageously begins only after such work is completed. We are ready whenever possible to facilitate coöperation between the scientist with ideas for development and selected manufacturers with facilities applying thereto. We own no patents, have part in no monopolies and all of the merchandise offered herein is obtainable either directly from the makers or through other dealers whenever our services fail in their operation toward the convenience, economy and general satisfaction of the purchaser."

This creditable piece of book-making bears the imprint of The Waverly Press Baltimore.

# ON THE WEIGHT OF SOME OF THE DUCTLESS GLANDS OF THE NORWAY AND OF THE ALBINO RAT ACCORDING TO SEX AND VARIETY

SHINKISHI HATAI

*The Wistar Institute of Anatomy and Biology*

FIVE CHARTS

## INTRODUCTION

In connection with another investigation, it was found that in the albino rat some of the ductless glands show a distinct sex difference in weight (Hatai '13). It was found later that a similar sex difference occurs in the Norway rat also. When, however, these two forms of rats are compared, the weights of the ductless glands are again in most instances characteristic for each form. In view of the fact that the albino rat is the domesticated variety of the Norway rat, the differences thus presented appear highly interesting and suggest a somewhat new line of investigation. It therefore seems worth while to note briefly the weight relations of these ductless glands in the two forms of rats, using the data which are available at the present moment.

The ductless glands with which we deal here are the suprarenals, hypophysis, thymus, thyroid, testes and ovaries. A part of the Norway records here used was obtained by Dr. C. M. Jackson while he was at the University of Missouri. He has kindly placed his entire data at my disposal and I take this opportunity to thank him for his courtesy in this matter. For the weights of the ductless glands in the albino rat, the reader is referred to my recent papers (Hatai '13, '14). The original individual data are deposited in The Wistar Institute of Anatomy in Philadelphia, where they may be consulted by anyone interested.



## MATERIAL AND METHODS

1. *The suprarenal glands*

The weight relations between the body and the glands in both the Norway and albino rats are shown in table 1 and their graphic representation in chart 1.

*a. Albino rat.* As is shown in chart 1, for a given body weight the weight of the suprarenal glands of the male albino rat is less

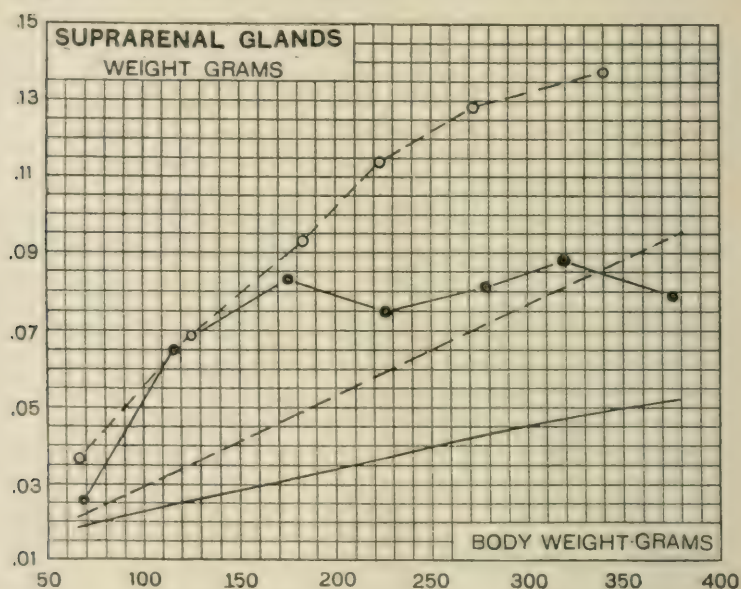


Chart 1 Showing the weight of the suprarenal glands in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat.

Males ● — ● Norway, observed ○ - - ○ Females  
Males — — Albino, calculated - - - Females

than that of the female. This sex difference becomes greater as the rat grows in weight. Furthermore, the difference appears at an early period of life; indeed it is already obvious at about 35 days of age, while sexual maturity is seldom attained in these rats before 60 to 90 days.

The sex difference in the weight of the suprarenals in the albino rat is thus not primarily connected with pregnancy in which con-

dition the female suprarenals are considered by some investigators (Biedl '13, and Vincent '12) to undergo hypertrophy.

*b. Norway rat.* As is shown in chart 1, the suprarenal glands of the Norway rat exhibit similar sex differences. Furthermore, the glands of the Norway rat are considerably heavier than those of the albino. We have not yet determined in the Norway rat the exact time of the appearance of the sex difference of this gland.

In table 1 we notice that the sex difference in the weight of the suprarenal glands is on the average 35 per cent in the Norway

TABLE 1

*Showing the weights (grams) of the suprarenal glands in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat*

SUPRARENAL GLANDS							
MALES				FEMALES			
Body weight	No.	Norway observed	Albino calc.	Albino calc.	Norway observed	No.	Body weight
69	1	0.026	0.018	0.021	0.037	4	67
117	4	0.065	0.025	0.035	0.069	3	126
175	5	0.083	0.031	0.049	0.093	6	183
226	17	0.075	0.037	0.059	0.109	15	224
278	15	0.081	0.042	0.070	0.128	10	272
319	10	0.088	0.047	0.086	0.137	5	340
375	1	0.079	0.052				
Avg. 223	53	0.071	0.036	0.053	0.096	43	202

and 47 per cent in the albino rat, both in favor of the females. However, owing to the deficiency of 21 grams in body weight of the female as compared with the male, some correction for the percentage differences just obtained, should be made.

By graphic interpolation from chart 1, we find that the weight of the female suprarenals in the Norway corresponding to 223 grams of body weight is nearly 0.109 gram. When this interpolated value for the female is compared with that observed for the male, we find a difference of 54 per cent in favor of the female rat. Similarly, we find a difference of 61 per cent in the albino rat in favor of the female.

TABLE 2

*Showing the weights (grams) of the hypophysis in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat*

HYPOPHYSIS							
MALES				FEMALES			
Body weight	No.	Norway observed	Albino calc.	Albino calc.	Norway observed	No.	Body weight
186	1	0.0065	0.0071	0.0123	0.0071	4	182
226	14	0.0071	0.0082	0.0157	0.0086	9	225
281	15	0.0085	0.0097	0.0195	0.0095	4	273
315	1	0.0100	0.0107				
Avg. 252	31	0.0080	0.0089	0.0158	0.0084	17	227

Concerning the differences between the Norway and albino rats in regard to the weight of the suprarenals, we find the following relations:

The suprarenals of the male Norway rat are heavier than those of the male albino rat by 97 per cent.

The suprarenals of the female Norway rat are heavier than those of the female albino rat by 80 per cent.

On the average, we obtain 89 per cent in favor of the Norway rat. We conclude therefore that the Norway rat, both sexes combined, possesses suprarenal glands which are nearly twice as heavy as those of its domesticated albino variety.

This difference in the weight of the suprarenals between the Norway rat and its albino variety has already been noted by Watson ('07) but he did not distinguish the sexes. Watson's observations were made on suprarenals which had been preserved in formalin.

The sex difference in the suprarenals is shown not only by their weight, but also often by their colors. For instance, in the albino rat the suprarenals of the male are a deep olive in color, while those of the female are much lighter. In the Norway rat, on the other hand, the color of the glands is ashy white in both sexes.



## 2. The hypophysis

The weight relation between the hypophysis and the body in both the Norway and albino rats is shown in table 2, and its graphic representation in chart 2.

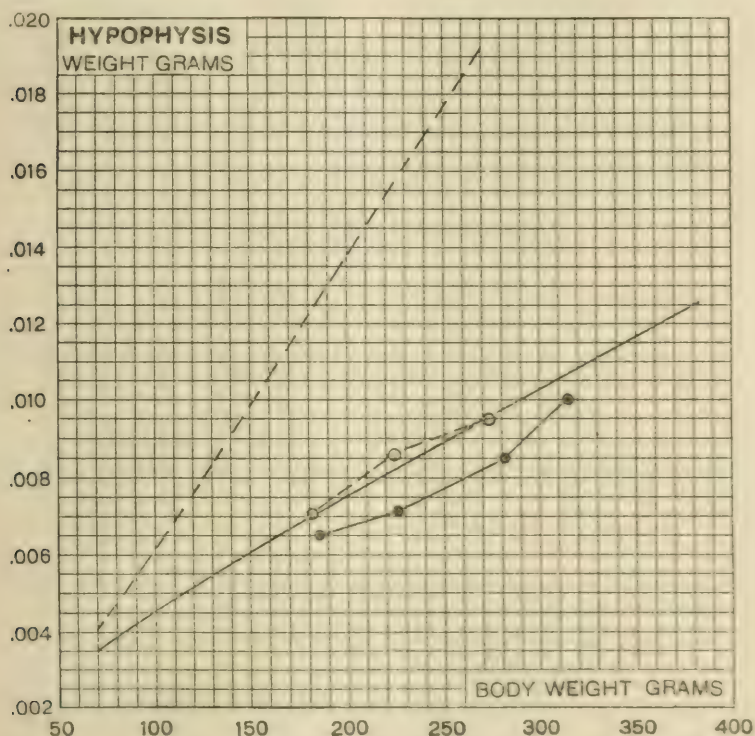


Chart 2 Showing the weight of the hypophysis in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat.

Males ● — ● Norway, observed ○ --- ○ Females  
Males — Albino, calculated --- Females

*a. Albino rat.* The sex difference in the weight of the hypophysis is more striking than in the case of the suprarenal glands, and indeed the difference, after a proper correction for the difference in body weight in the two sexes has been made, amounts to 97 per cent in favor of the female rat. The difference appears at about 30 to 40 days of age and thus is not primarily associated

with pregnancy in the female, during which condition the hypophysis is assumed to undergo hypertrophy.

*b. Norway rat.* Curiously, the sex difference in the weight of the hypophysis in the Norway rat is considerably smaller, and furthermore, the weight of the hypophysis in both sexes is smaller than in the corresponding albinos. The difference in the weight of the hypophysis in the Norway rat is found in the following way: From the graph for the female hypophysis in chart 2 we obtain a weight of about 0.0092 gram, corresponding to 252 grams of observed male body weight. When this value of the female hypophysis is contrasted with 0.008 gram for the observed male hypophysis (see table 2, average for male), the difference is 15 per cent in favor of the female rat.

Although the difference of 15 per cent is quite small when compared with that of 97 per cent, shown by the albino variety, nevertheless its reality is evident from the regularity and uniformity of the results shown in chart 2. It is interesting to note that the hypophysis of the Norway shows not only a small sex difference, but its absolute weight is considerably less than in the corresponding sexes of the albino rat. We obtain from table 2 the following relations:

The weight of the hypophysis of the male Norway is less than that of the male albino rat by 11 per cent.

The weight of the hypophysis of the female Norway is less than that of the female albino rat by 46 per cent.

We may note from the above relations that the smaller sex difference shown by the hypophysis in the Norway as contrasted with the albino, is especially due to the relatively smaller hypophysis of the Norway female. The sex difference is shown also in the general appearance of the hypophysis.

In both the Norway and albino rats the hypophysis of the female is much swollen, the upper surface is more convex and the color is a deeper pink than in that of the male. However, we do not find any characteristic appearance distinguishing this gland in the Norway from that in its albino variety.

### 3. The thyroid gland

The weight relation between the thyroid and the body is given in table 3, and its graphic representation in chart 3.

a. *Albino rat*. Unlike the suprarenals and hypophysis, the thyroid gland of the albino rat does not exhibit any difference distinguishing the sexes either in weight or in appearance. It must be admitted, however, that this failure to reveal a sex difference may be due either to its absence, or to the fact that the sex difference may be masked by the great variability of the thyroid. With our present data the variation in the weight of the thyroid in the albino rat according to sex is not ascertainable (Hatai '13).

TABLE 3

*Showing the weights (grams) of the thyroid gland in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat*

THYROID GLAND							
MALES				FEMALES			
Body weight	No.	Norway observed	Albino calc.	Albino calc.	Norway observed	No.	Body weight
69	1	0.015	0.014	0.015	0.014	3	73
117	4	0.022	0.021	0.022	0.025	2	122
174	4	0.029	0.029	0.030	0.034	6	183
226	17	0.033	0.035	0.035	0.028	15	224
278	15	0.031	0.042	0.041	0.042	10	272
319	10	0.050	0.046	0.049	0.078	3	342
375	1	0.046	0.053				
Avg. 223	52	0.032	0.034	0.032	0.037	39	203

b. *Norway rat*. In the Norway rat also the variation in the weight of the thyroid is considerable. Thus the slight excess shown in the weight of the female thyroid (table 3) is difficult to interpret. However, from the general trend of the graph, the difference here noted may be an incidental one. Further, it is an interesting fact that the weight of the Norway thyroid is practically identical with the weight of the albino thyroid.

Although I am unable to trace the authority for the statement, the thyroid gland in man is the only ductless gland which is



usually stated in the anatomical text books to exhibit a sex difference in weight. As we see, however, the thyroid gland of the rat not only fails to exhibit a sex difference, but fails also to respond to the changes of environment represented by domestication. If, therefore, our information concerning the human thyroid be correct, we have here an interesting difference in the comparative anatomy and physiology of this gland.

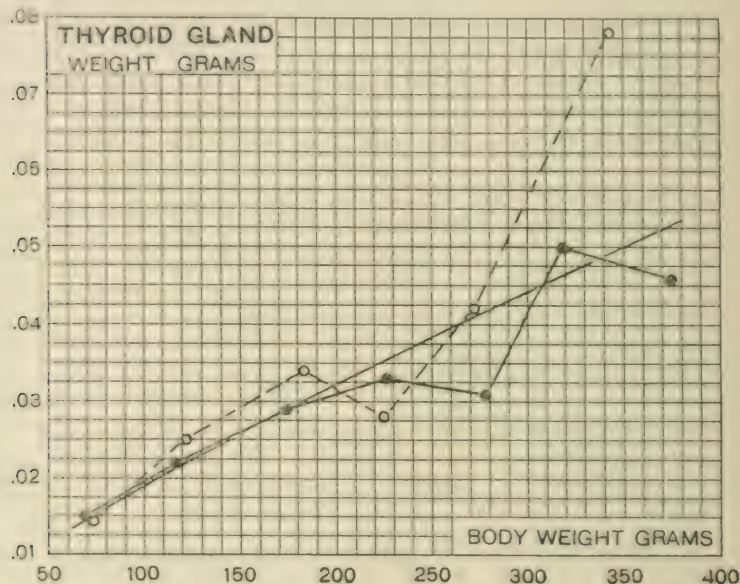


Chart 3 Showing the weight of the thyroid in the two sexes of the Norway compared with those in the albino rat.

Males ● — ● Norway, observed ○ --- ○ Females  
Albino, calculated — Both sexes.

#### 4. The thymus gland

The weight of the thymus gland is correlated with the age of the animal and is not evidently different according to sex (Hatai '14). Since our data for the Norway rat lack age records, no legitimate comparison between the Norway and albino thymus can be made. Consequently, the data on the weight of the thymus are excluded from the present paper.

### 5. The sex glands

The weight relation between the body and sex glands in both the Norway and albino rats is given in table 4, and its graphic representation in charts 4 and 5.

a. *Testes of the Norway rat as compared with those of the albino rat.*  
The weight of the testes of the Norway rat is considerably greater

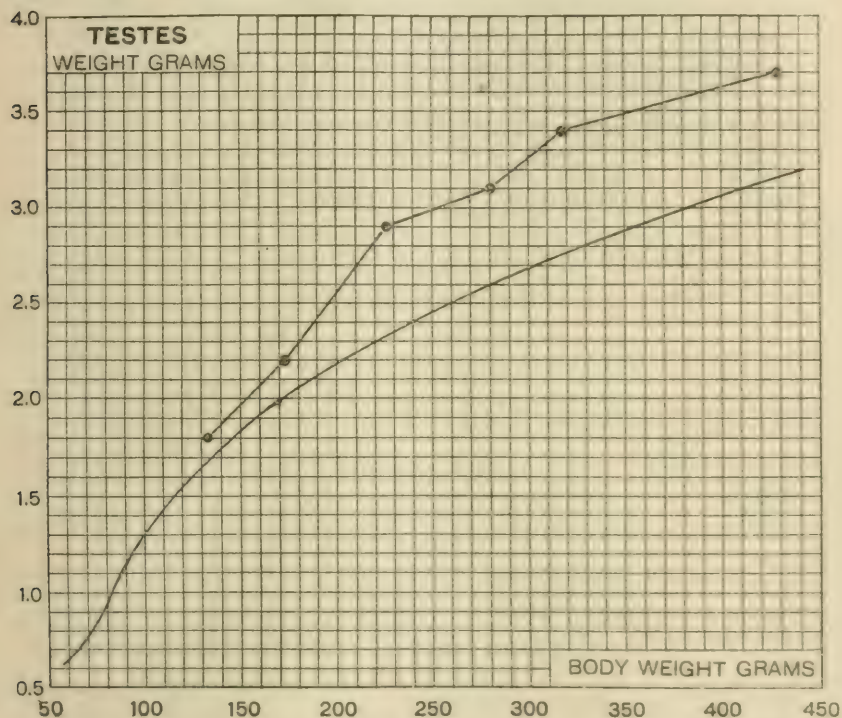


Chart 4 Showing the weight of the testes of the Norway rat compared with that of the albino rat.

Norway, observed ● — ● Albino, calculated —

for the same given body weight, than in the albino rat. The difference amounts to 21 per cent in favor of the Norway. I am unable to state at present whether this excess of 21 per cent is due to a uniform enlargement of all the structures of the testes, or whether it is due to the enlargement of some particular constituent. The histological investigation of this point will be of interest.

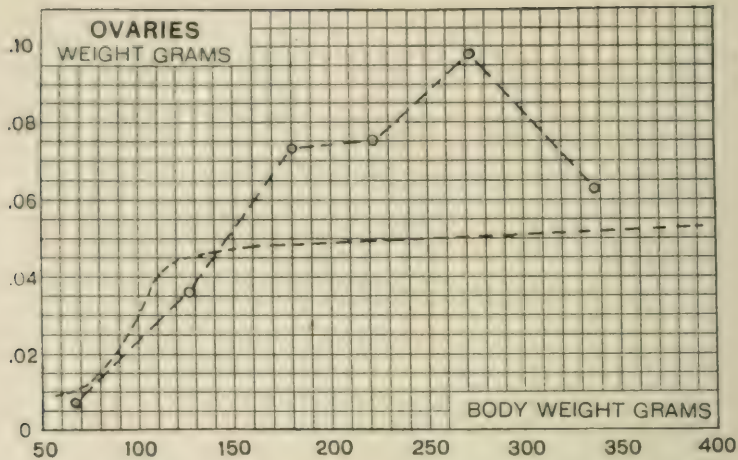


Chart 5 Showing the weight of the ovaries of the Norway compared with that of the albino rat.

Norway, observed ○ --- ○ Albino, calculated - - - -

*b. Ovaries of the Norway rat as compared with those of the albino.* For the same body weight, the ovaries in the Norway rat are considerably heavier than those in the albino. The difference amounts to 26 per cent in favor of the Norway. For the ovaries also we have no histological data as to the structures that are responsible for this excess in weight.

TABLE 4

*Showing the weights (grams) of the sex glands—testes and ovaries—in the two sexes of the Norway compared with those in the corresponding sexes of the albino rat*

SEX GLANDS							
MALES—TESTES				FEMALES—OVARIES			
Body weight	No.	Norway observed	Albino calc.	Albino calc.	Norway observed	No.	Body weight
				0.010	0.007	4	67
133	5	1.8	1.7	0.045	0.036	5	126
172	11	2.2	2.0	0.048	0.073	7	180
226	16	2.9	2.3	0.050	0.075	21	221
280	17	3.1	2.6	0.051	0.098	12	272
317	8	3.4	2.8	0.052	0.063	6	337
429	10	3.7	3.1				
Avg. 260	67	2.9	2.4	0.043	0.059	55	201



## DISCUSSION

The preceding analysis shows that with the exception of the thyroid and the thymus, the weight of the ductless glands in the two forms of rats exhibits (1) a difference according to sex and (2) a difference according to zoological variety.

*1. Difference according to sex*

Although there are scattered statements concerning some of the ductless glands for man, I am not aware that the sex relations of these glands has been previously thus clearly shown. At the same time, even in recent studies of the glands, both in man and animals, the sexes are sometimes either combined or not given. Consequently, in the majority of cases, information with regard to the sex relations cannot be obtained. Whether or not the sex difference in these ductless glands is as marked in other animals as in the rat, remains to be determined.

Elliott and Tuckett's work ('06) suggests strongly the existence of a sex difference in the weight of the suprarenals in guinea-pigs, rabbits and cats. The amount of data given by these authors, however, is not sufficient for a critical test on this point. Recently Kolmer ('10) noted the structural difference in the suprarenals of guinea-pigs according to sex. It thus appears that so-called "hypertrophy of some of the ductless glands" in the females during pregnancy or during other special physiological conditions, must be received with reservation until data on the possible sex difference of the normal individuals have been obtained.

*2. Difference according to zoological variety*

This is another interesting relation quite worthy of further careful investigation. We have no data for animals other than rats showing the weight of the ductless glands in zoological varieties. Watson ('07) first noted that the suprarenals of the Norway rat are always heavier than those of the albino rat. The present investigation fully supports Watson's finding. Watson ('08) further noted that Norway rats under captivity lose in the

weight of the suprarenals as much as 28 per cent (computed from the absolute weight) within the first ten weeks. Unfortunately Watson did not record the sexes and consequently, since the weight of the adrenals show nearly 54 per cent normal difference according to sex, the reported reduction of 28 per cent cannot be accepted without reservation until it has been confirmed on rats of the same sex.

Elliott and Tuckett ('06) notice the weight variation in the suprarenals of different strains of guinea-pigs. It seems highly probable that investigations along this line might throw some light on the physiology of these interesting members of the endocrine system.

I shall not attempt at this time to interpret any of the differences observed according to either sex or variety; nevertheless, it may be stated in regard to the difference found between the two forms of rats that such differences have appeared to be the result of a response to the complex conditions represented by domestication. If it should appear that similar changes took place in other species under domestication, we would have an important instance of adaptation within the organism to the changes in the environment.

#### CONCLUSIONS

1. In both the Norway and albino rats the suprarenal glands of the males are considerably smaller than those of the females. When, however, these two forms of rats are compared, both sexes of the Norway rats have suprarenals considerably heavier than those of the like sexes of the albino.

2. A sex difference is noted in the weight of the hypophysis in both the Norway and albino rats. The male hypophysis is lighter than that of the female. However, when these two forms of rats are compared, the hypophysis of the Norway is found to be smaller than that of the albino rat; the greater difference being in the case of the female.

3. Neither in the Norway nor the albino rat is a sex difference found in the weight of the thyroid. Moreover, there is no weight

difference in the thyroid according to variety in these two forms of rats.

4. The sex glands (testes and ovaries), of the Norway rats, are heavier than those of the albino rats.

5. The differences found between the Norway and albino rats with respect to the weight of the ductless glands seem to be the result of a response to the complex conditions represented by domestication.

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Notice is again given to all members wishing to present a communication at the St. Louis meeting that an abstract of the communication of not more than four printed pages in length must be in the hands of the secretary before December 5th. Members expecting to show demonstrations are also requested, where it is possible, to write a short description of the demonstration and send it in before December 5th, in order that this also may be published in the Proceedings.

Professor R. J. Terry, Washington University Medical School, St. Louis, Mo., should be communicated with regarding the facilities needed for a particular demonstration, as well as for directions, etc., as to how to ship material and apparatus to be shown at the meeting.

A plan has been arranged to supply every member attending the Thirty-first Session, December 28, 1914, with a set of page proofs of Abstracts, Titles and Demonstrations of the Scientific Program to be presented. Proofs cannot be sent to authors. The copy sent in should therefore be clear, correct and in shape to be put in type exactly as written. Corrections may be marked on the sets of proofs supplied at the meeting. These corrections will be made before publication in "The Anatomical Record."

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*November 9, 1914*

# ON THE MECHANISM OF MORPHOLOGICAL DIFFERENTIATION IN THE NERVOUS SYSTEM

## I. THE TRANSFORMATION OF A NEURAL PLATE INTO A NEURAL TUBE

OTTO C. GLASER

*From the Zoological Laboratory of the University of Michigan*

THREE FIGURES

### I. INTRODUCTION

The early development of the vertebrate nervous system is among the commonplaces of descriptive embryology. Every elementary text-book tells us that the first clearly recognizable rudiment is a flat patch of cells ectodermal in nature; how in due course of time after the appearance of a longitudinal furrow, the edges of this plate rise, meet in the mid-dorsal line, and fuse to form a tube, enclosing the neurocoel. What however are the forces at work when the primitive plate changes into a tube?

This question was in the mind of His<sup>1</sup> when he wrote his classic letters, "Unsere Körperform." In the fourth of these lucid epistles, His shows that the nervous system, during the period of folding, grows faster than the surrounding tissues with which it is continuous. On the assumption that these resist any increase in the width of the neural plate, he shows that the latter, must fold under the mechanical necessities of the case. Models, in which the entire process could be simulated at will, helped to emphasize the argument.

The well-known experiments of Roux,<sup>2</sup> however, proved that this view of the origin of the neural groove is mechanically impossible, despite the inequalities of growth emphasized by His.

<sup>1</sup> *Unsere Körperform, und das Physiologische Problem Ihrer Entstehung.* F. C. W. Vogel, Leipzig, 1874.

<sup>2</sup> *Die Entwicklungsmechanik.* W. Engelmann, Heft 1, Leipzig, 1905.

Roux indeed was able to produce a fold in the neural plate of the chick by pressure from the sides, but when this pressure was released, the plate instantly returned to its original flat condition. Pressing upon it through the lateral extra-neural membranes, these instead of transmitting the pressure, collapsed, a result which might have been foreseen when their thickness is compared with that of the plate (*loc. cit.*, p. 45).

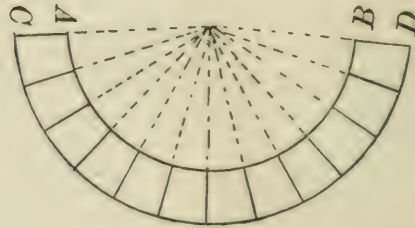
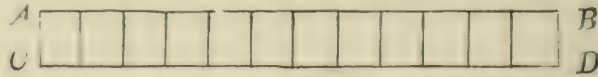


Figure 1

Most conclusive have been Roux's isolation experiments. By cutting the neural plate from its surroundings he found that it still folded in the normal manner, "*und zwar geschah dies noch rasher als es normalerweise der Fall ist*" (*loc. cit.*, p. 451). Folding occurred even when in addition to lateral isolation, the neural plate was cut transversely into a number of segments. From these experiments the conclusion was drawn that the nervous system is self-differentiating.

Not only do the lateral membranes, as implied in the remark quoted, contribute nothing toward converting the plate into a tube, but they are actually an hindrance, for they exert a pull away from the mid-dorsal line where fusion of the neural folds finally takes place. I have convinced myself by a few simple experiments on the embryos of *Amblystoma punctatum*, that such pull away from the median axis actually exists. In these,



if a longitudinal cut be made in the neural plate, the wound gapes widely for twelve hours or more if the extra-neural ectoderm has been uninjured, but if this is also cut on each side of the nervous system parallel with the incision in the plate, the wound in the latter after twelve hours, is very much smaller than in the first experiment, or has practically disappeared.

If we accept, as it seems to me we must, Roux's conclusion that the nervous system is self-differentiating, we must ask what this statement means. Evidently for the stages of development under consideration here, self-differentiation is identical with self-folding. The question to be answered therefore is how the neural plate can autonomously fold itself.

## II. THE CHANGE IN THE SHAPE OF THE CELLS DURING FOLDING

Rhumbler's<sup>3</sup> very complete analysis of the geometrical relations in invaginate gastrulation finds its application to the case in hand. For the sake of simplicity let us imagine it possible to cut through a neural plate a rectangular cross-section,  $ABCD$ , in which each component cell is itself a rectangle. If this section is folded symmetrically about an axis,  $AC$  and  $BD$ , and all cell-boundaries parallel with them, will radiate toward a center, and  $AB$  and  $CD$  will become respectively the circumferences of two spheres, one with the radius,  $R$ , the other with the radius,  $R + AC$ . It follows from the geometrical necessities of the case, not only that circumference  $CD$  is greater than  $AB$ , but that each cell, from being a rectangle, has become a trapezium whose lower boundary is greater than its upper.

Although actual conditions in the nervous system are more complicated, nevertheless, sections through the two stages under consideration approximate the ideal case very closely. An examination of any section, or any one of the thousands of published figures, will disclose many cells in which the change in shape here emphasized is strikingly shown. Indeed, being a geometrical necessity, the case cannot be otherwise, but whether the change in shape is the result of folding, or folding the result

<sup>3</sup> Zur Mechanik des Gastrulationsvorganges; Arch. f. Entwicklungsmech., Bd 14

of the change in shape, remains unanswered. One thing however appears certain; we must seek the answer in the nervous system itself, for the neural plate is self-folding.

### III. THE NUMBER OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS




In order to discover how the neural plate folds itself, we must first find what rôle, if any, cell-division plays in the process. Determinations capable of answering this question could be made if we could count the number of cells in comparable sections at different stages in the development of the tube. However, the nervous system is so largely syncytial in nature, and cell-boundaries, even where present are often so ill defined, that this direct method would surely lead to error. Another road is open however, for we may count accurately the number of nuclei. For this purpose the most satisfactory material I have found are some embryos of *Cryptobranchus allegheniensis*, for the possession of which I am indebted to Prof. Bertram G. Smith of the Michigan State Normal College.<sup>4</sup>

The period of folding was arbitrarily divided into three, beginning with the flat neural plate, and ending with the neural tube just prior to fusion. Between these extremes is the half-folded plate. For the stages in question I selected ten comparable but un consecutive sections from the middle point of each series. The sections were all 10 micra in thickness, and the number of nuclei in each was carefully counted. The results are given in table 1, in which each column is headed by a diagram representing the stage of development referred to. Examination of this table indicates that during folding the number of nuclei, and hence of cells per section, does not increase. At the beginning of the period there are, on the average, 62 cells per section, in the middle, 61, and at the end, 59.

<sup>4</sup> For details on the development of *Cryptobranchus*, not dealt with in the present paper, see the excellent communications of B. G. Smith: Biol. Bull., vols. 11 and 26, and Journ. Morph., vol. 23.

Naturally these values are not absolute. Lack of uniformity in the distribution of nuclei in the syncytial system is responsible for considerable variations in individual sections, and may have affected the averages. Some errors no doubt have crept in on account of faulty enumeration, and also because the sections are necessarily from different individuals. The seriousness of the first source of uncertainty seems to me largely offset by the remarkable constancy of the averages; against the second source

TABLE I  
*Number of nuclei in comparable sections*

STAGE I		STAGE II		STAGE III	
					
	63		56		55
	53		64		60
	58		50		73
	69		56		47
	72		50		69
	58		82		59
	59		70		64
	58		74		51
	58		58		52
	68		51		55
Ave.	62		61		59

of error I guarded by reflecting the nuclei on paper, dotting each one as counted, and then recounting the dots as a check; the third difficulty was met as completely as possible by choosing embryos from eggs of uniform size laid by a single female.

The conclusion that cell division or better, multiplication, does not occur during the process of folding, although possibly correct, cannot be drawn without a certain reservation, for the nervous system increases in volume during this period (see Section V). With this fact in view one cannot consider the constancy in the number of nuclei per section sufficiently conclusive to warrant the statement that cell multiplication does not occur



during the period of folding, and hence can play no part in transforming the neural plate into a tube. However if it does occur, the number of nuclei produced in this way is too small to overcome the 'nuclear dilution' brought about by the volumetric increase of the system as a whole. Relatively, therefore, even if not actually, the number of cells per section remains constant, and we can make no great mistake by assuming that the rôle of cell-multiplication is practically negligible.

#### IV. THE DISTRIBUTION OF NUCLEI IN COMPARABLE SECTIONS OF THE NERVOUS SYSTEM AT THE BEGINNING AND AT THE END OF THE FOLDING PROCESS

If we mark off a series of points midway between the upper and lower surfaces of the neural plate, a line connecting them will divide the nervous system into an upper and a lower zone. A corresponding line drawn in the half, or fully, folded stages, gives us inner and outer zones. Since morphologically upper and lower are identical with inner and outer respectively, it will be advantageous to use the latter terms also for the unfolded neural plate.

The nuclei of the sections which served as the basis for table 1, are distributed in the inner and outer zones in the proportions given in table 2. Comparing the three stages, we find that the distribution of nuclei in the inner and outer zones is quite different at the beginning and at the end of the folding, and that Stage II occupies an intermediate position. Roughly, the inner zone loses one-half its nuclei and the 'nuclear concentration' in the outer zone increases by this amount. Absolute correspondence between the nuclear loss of one zone, and the gain in the other, cannot be expected. Not only must we recall the sources of error mentioned in section III, but we must also remember that the division into inner and outer zones is a somewhat arbitrary expedient, and furthermore that the neural plate, although spoken of as flat in Stage I, is in reality quite irregular in detail, and moreover exhibits, more or less, the general curvature of the sphere of which it is a part. Without attempting any special

refinements which seem to me quite unnecessary, it is obvious that during folding there is a marked outward migration of nuclei (see text-figure 2). With this outward migration, there is associated, from the geometrical necessities of the case, a distinct increase in the volume of the outer zone. Both these changes in the folding nervous system would occur if this tissue were suitably pressed upon from without, but since the neural plate folds itself, the forces that result in the translocation of materials and the change in the shape of the cells must be sought within the autonomous system.

#### V. THE INCREASE IN THE VOLUME OF THE NERVOUS SYSTEM DURING FOLDING

Change of shape in the cells of a folding tissue may occur with constant volume. In the neural plate, for instance, the inner zones of the cells might decrease by an amount exactly equalled by the increase in the outer zones. This, however, is not true.

TABLE 2  
*Distribution of nuclei in inner and outer zones*




STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
32	31	31	25	15	40
32	21	22	42	15	45
34	24	16	34	21	52
55	14	27	29	13	34
39	33	18	32	22	47
38	20	27	55	16	43
31	28	33	37	26	38
33	25	29	45	13	38
37	21	21	37	20	32
44	24	19	32	20	35
Ave. 38	24	24	37	18	39



Fig. 2 Two sections through the embryonic nervous system of *Cryptobranchus allegheniensis*, showing the nuclear distribution in Stages I and II. The sections are from the same series and regions as those dealt with in the tables but contain for Stage I, six, and for Stage II, one nucleus more than the maximal number recorded in table 1. In the unfolded plate there are in the present case, 78 nuclei, of which 47 are in the inner zone, and 31 in the outer; in the half-folded plate, there are 75 nuclei, 21 in the inner zone, and 54 in the outer. Nuclei which happen to fall on the line separating the two zones are ascribed to the one into which the greater portion of their mass projects.

Adequate measurements cannot be carried out directly. Instead, we may compare the areas of the two regions, in section, for area is definable as volume in one plane. On this basis, determinations capable of giving some insight into the volumetric relations during involution are easily made; all that is needed is to draw the sections at constant magnification with the aid of a camera lucida, and then by means of a planimeter, trace the relative areas of the inner and outer zones. The results of such measurements, carried out on the same sections used in making the nuclear counts, are presented in table 3. From this table it is apparent that the areas of the two zones are related during the process of folding by the following ratios:

	STAGE I	STAGE II	STAGE III
Inner zone.....	4.8	4.3	7.4
Outer zone.....	4.4	6.8	12.3






In other words, while inner and outer zones are approximately equal in Stage I, in Stage II, the outer zone exceeds its original size by half, and the inner remains practically unchanged. In Stage III on the other hand, both zones show an increase, but the inner, roughly, has doubled, the outer, trebled, its volume. Although it is impossible under these circumstances to determine how much of the increase in the volume of one zone is due to the migration of nuclei and cytoplasmic materials from the other, it by no means follows that the outer zone does not gain at the expense of the inner. Indeed such translocation is definitely proved in the case of the nuclei, and appears inevitable for the other cell contents.

#### VI. ON THE CAUSE OF THE INCREASE IN VOLUME

Since cell-multiplication during the process of folding appears negligible, it cannot be concerned practically with the increase in volume which takes place at this time. It follows that the cells of the nervous system must individually increase in volume.

TABLE 3  
*Relative areas of inner and outer zones*

STAGE I		STAGE II		STAGE III	
					
Inner	Outer	Inner	Outer	Inner	Outer
4.6	4.8	5.3	8.4	6.6	10.9
4.4	3.7	4.1	7.2	6.9	12.6
6.7	3.9	4.6	6.8	6.9	12.6
5.2	4.7	4.3	5.8	7.8	12.8
5.0	4.1	4.1	6.0	7.3	13.2
4.8	4.8	4.0	6.9	6.8	11.3
4.6	4.5	5.0	6.5	7.7	11.3
3.3	4.9	4.2	7.8	7.7	10.8
4.4	3.9	3.7	6.5	7.3	11.6
4.9	5.0	3.8	6.3	8.5	10.3
Ave. 4.8	4.4	4.3	6.8	7.4	12.3

and this might be due to the absorption of water. Such absorption would be capable of direct demonstration if it were possible to compare the water content of the neural plate with that of the neural tube, but very serious obstacles of a technical nature stand in the way of the required determinations. It would be exceedingly difficult to isolate neural plates in sufficient numbers, or with sufficient accuracy, to make the necessary weighings reliably. Another method of procedure is open.

The rate of growth in these early stages of the nervous system is known to differ from that of the surrounding tissues. Since growth in general is measurable in terms of water absorption it follows that the differential growth of the neural plate and tube must be the reflection of a differential absorption of water on the part of their component cells. If this is correct then at the stage of complete involution, the water content of the neural tube should differ from the water content either of the larva, taken as a whole, or of certain portions, for unless this were true, the embryo, instead of having grown in complexity, would simply have grown in size.

The most satisfactory material available for the study of this question proved to be the eggs of the frog, *Rana pipiens*, and of the salamander, *Amblystoma punctatum*. Development in both cases was allowed to proceed normally until the bodies of the embryos could be conveniently cut from the yolk-sac, an operation easily carried out with a minimal loss of tissue. Unfortunately the separated portions cannot be further divided, and even if the isolation of their constituent layers were possible the inclusion of yolk within the cells of the nervous system would leave an uneliminated and unavoidable error. However it is safe to assume that our operation results in the separation of two tissue masses, one predominantly yolk, the other predominantly nervous.

The fresh weight of the entire larvae as well as that of the separated tissue masses was in each case ascertained after carefully removing the superficial water. Following this, dry-substance determinations were made in the usual manner by complete dessication at 60°C. *in vacuo*, over  $P_2O_5$ . For the sake of easy

comparison, all the results are assembled in table 4. From this table it is at once apparent that the water content of the frog and salamander embryos at this stage of development belong to the same order of magnitude, and further that the water content of the yolk-sac of the frog larvae is not very different. Corresponding determinations for the yolk-sac of *Amblystoma* proved impracticable as the consistency of the yolk in these embryos is such that considerable losses occur when the sac is removed. However, the values for the entire larvae are identical in the two cases.

Comparing the embryonic nervous systems of these two forms with the entire larvae or the yolk-sacs, it is seen at once that the water content of the first belongs to a totally different order of magnitude, for the nervous system is a tissue which, within the limits of error may be said to contain 80 per cent of water and 20 per cent of dry substance.

TABLE 4

*Showing water content four to five days after fertilization*

MATERIAL	FRESH WEIGHT	DRY WEIGHT	DRY SUBSTANCE	WATER
	grams	grams	per cent	per cent
R. Pipiens				
38 Larvae.....	0.1278	0.0557	43.8	56.2
50 Larvae.....	0.1718	0.0722	42.0	58.0
39 Larvae.....	0.1815	0.0630	40.2	59.8
Average.....			42.0	58.0
24 Yolk-sacs.....	0.0440	0.0204	46.4	53.6
31 Yolk-sacs.....	0.0585	0.0264	45.2	54.8
Average.....			45.8	55.2
24 Nervous systems.....	0.0464	0.0098	19.1	80.9
31 Nervous systems.....	0.0714	0.0149	20.8	79.2
Average.....			19.9	80.1
A. Punctatum				
16 Larvae.....	0.0955	0.0399	41.8	58.2
15 Larvae.....	0.0992	0.0406	40.9	59.1
Average.....			41.4	58.6
125 Nervous systems.....	0.3914	0.0785	19.9	80.1
52 Nervous systems.....	0.1756	0.0400	22.8	77.2
15 Nervous systems.....	0.0524	0.0106	20.2	79.8
69 Nervous systems.....	0.2039	0.0363	17.8	82.2
Average.....			20.2	79.8



The essential correctness of these values for the nervous system is guaranteed by the identity of the averages, the relative constancy of the individual observations, and finally by comparison with the adult condition. This comparison<sup>5</sup> can be made in the case of *Rana pipiens*, because Donaldson<sup>6</sup> has given us certain standard values for this form. In making this comparison it is more correct to use the water content of the adult cord, for this is less differentiated than the brain and hence more nearly in the larval condition. According to Donaldson's figures (*loc. cit.*) the average water content of 12 cords of *Rana pipiens* is 80.5 per cent, a value identical with mine of 80.1 per cent for the larval system.

Since the rate of cell-multiplication during folding is either zero or practically negligible; since this period, moreover, coincides with a great increase in volume, and finally, since the water content of the neural tube differs so radically from that of either the yolk-sac or the larva taken as a whole, it seems scarcely doubtful that the differential absorption by the nervous system occurred during the process of involution. This, however, does not prove that the differential absorption is responsible for the folding.

#### VII. ON THE THEORY OF FOLDING

Since every metazoan body arises from germ-layers having a common origin in the egg, and connected with one another without interruption, it follows that even a complicated organism, theoretically at least, could be unravelled and spread out in the form of a continuous membrane. It has been evident for many years that in embryogenesis, the commonest occurrence leading to increased complexity of form, is the folding process, but its causes have not been adequately analysed, nor have attempts at analysis received the recognition from anatomists and embryologists, which they deserve.

<sup>5</sup> Otto Glaser, The water content of the embryonic nervous system. *Science*, vol. 39.

<sup>6</sup> Donaldson, Henry H., Further observations on the nervous system of the American leopard frog, *Rana pipiens*, etc. *Jour. Comp. Neur.*, vol. 20; see also numerous earlier papers.

From the standpoint of static morphology, the analysis of an organism into a system of folds may appear satisfactory enough, and constitutes a step forward. From the same standpoint, however, every fold is like every other, a complication, the contemplation of which can give us no idea as to how it was produced. From the dynamic point of view, the mode of production is the important thing, and a moment's reflection is enough to show that significance of one fold may be quite different from that of another.

Experimental analysis of the early stages of the nervous system has gone far enough to show that here the folding process is autonomous. The same effect could be achieved by coercion, and would be morphologically indistinguishable. But coercion is a very different process from the one under consideration, and certain foldings of the heart and digestive system in the embryo, have only a superficial resemblance to the autonomous folding of the nervous system. Nevertheless, we are not without analogies, for it follows from Rhumbler's work (loc. cit.) that invaginate gastrulation belongs to the same category.

In the first place, Rhumbler draws attention to the significance of the change in shape undergone by the entoderm cells during infolding. Before as well as after gastrulation the cells are wedge-shaped, but in the blastula, the narrow ends of the wedges point inwards, as they do in all the other cells of the spherical larva, whereas during and after invagination the wedge-shape of the entoderm cells is completely reversed and their narrow ends now point outward. This change in shape, which has also been emphasized by Conklin,<sup>7</sup> occurs, as we have seen in the nervous system. According to Rhumbler, a translocation of materials within each of the involved cells is a mechanical necessity. "*Die Umgestaltung der Zellen der Entodermplatte, d. h. die Verbreiterung der Entodermzellenkeile auf der Blastocölseite, erfordert unbedingt Zellensubstanz-verlagerung innerhalb jeder einzelnen Entodermzelle nach der Blastocölseite hin, um verbreitern zu können was vorher zugespitzt war*" (loc. cit. p. 432).

<sup>7</sup> Mosaic development in Ascidian eggs. Jour. Exp. Zool., vol. 2, p. 163.

The migration of the nuclei which I have described is in strict harmony with this idea, and if the nuclei migrate it is probable that other portions of the cell contents also undergo a change in location.\* The occurrence of the same changes in shape on the part of the infolding cells in the two cases, however, is in itself not enough to show that invaginate gastrulation and the formation of a neural tube are fundamentally identical, for gastrulation by coercion would force upon the entoderm cells the shape which they would assume autonomously, if invagination were an autonomous process. There are cogent reasons for believing that this is true.

According to Rhumbler (*loc. cit.*, p. 410) there are conceivable three ways in which the entoderm cells might possibly be coerced from without. In the first place, differential growth on the part of the ectodermal and entodermal elements of the blastula might result in invagination; in the second place, the blastula, growing inside the egg-membrane, might have invagination forced upon it if the entodermal plate were less resistant to mechanical pressure than the ectoderm; and finally, the decrease in the volume of the blastocoelic fluid during invagination might result in a suction which would be followed by a caving-in of the weakest region in the wall.

The second and third possibilities are readily disposed of. It is only necessary to recall that invaginate gastrulation takes place perfectly well in the absence of an egg-membrane (Rhumbler). Furthermore, the entodermal plate cannot be the weakest region in the wall of the blastula since in general its component cells are the largest which the larva possesses. Mechanically the relation between the entoderm and the ectoderm of the blastula, must be the same as that between the neural plate and

\* In this connection, figure 2 in a recent paper by J. F. Gudernatsch in *The Anatomical Record* (vol. 7, p. 416) is interesting. In this picture the nuclei are located in the inner ends of the gastral plate cells *before* invagination. The author imagines that this localization renders the inner ends of the cells less compressible than the outer, and that invagination is caused by pressure exerted by the ectoderm on the more compressible outer ends of the gastral cells. It seems curious that the work of Rhumbler and Roux, mentioned in the very extensive bibliography attached to this paper, should have made so little impression.



the extra-neural membranes. Suction due to a decrease of the blastocoelic fluid would bring about the invagination not of the entoderm, but of the ectoderm.

Differential growth as a factor in invagination is not so easily disposed of. Rhumbler's analysis deals with the following possibilities:

*Case I.* Cell multiplication in the ectoderm proceeds at a higher rate than in the entoderm. The result is pressure upon the entodermal plate from the periphery, and this pressure may be sufficient to bend the plate. However, not only is this region of the blastula the least likely of all, to give way, but as long as the broad ends of the entodermal wedges point outward (Rhumbler) any folding produced by the means imagined would necessarily result in evagination, not invagination.

*Case II.* Cell multiplication in the entoderm proceeds at a higher rate than in the ectoderm. The effect would be the same as in Case I. A pressure on the entodermal plate would result, but no matter how produced, the plate could not fold inwards.

Consideration of these two possibilities of differential growth is worth while as an aid to clearing the ground, although Case II was unnecessary even at the time that Rhumbler wrote, for Morgan<sup>9</sup> had found that during gastrulation

Karyokinetic division is no more frequent in the cells involved than elsewhere. At the end of the period, if the posterior hemisphere be examined, it will be found that the plate of cells has disappeared from the surface, and that the surface nuclei are little more frequent than the surface nuclei of the anterior hemisphere. Karyokinetic division therefore plays no part in the development of the archenteron of the gastrula of *Sphaerechinus* (loc. cit., p. 85).

Although my conclusion with regard to the rôle of cell-multiplication during the folding of the neural plate is stated more conservatively, Morgan's result with respect to the same factor in the invagination of the gastral plate, is practically identical.

With respect to the change in shape, the consequent translocation of cell-contents, the influence of external pressure, and

<sup>9</sup> Studies of the 'partial' larvae of *Sphaerechinus*. Arch. f. Entwicklungsmech., Bd. 2.

finally, the rôle of cell-multiplication, invaginate gastrulation is identical with the folding process undergone by the nervous system. We may, therefore, safely attribute to the gastral plate an equal degree of autonomy.

But the elimination of cell division does not necessarily eliminate differential growth. We have already seen that an increase in the volume of the nervous system takes place at the time of folding, and that this growth, occurring with a negligible rate of cell-multiplication, is the result of water absorption. If such absorption were demonstrable for the gastral plate we should have one more point of identity between the two processes under comparison.

Unfortunately I have no trustworthy direct measurements. Nevertheless, there are considerations which seem to bear closely on the point at issue. Thus Morgan (*loc. cit.*, p. 85) says:

The nuclei in the walls of the archenteron enlarge during the invagination period and lie further apart than they did in the plate. This might give us some clue as to the mechanical principles involved in the process if we could estimate the volumes of the cells (protoplasm) before and after the process, but this it is impossible to do.

I have little doubt that the methods which I have employed for detecting the change in volume during the folding of the neural plate will be applicable to the process of invaginate gastrulation. What is of more immediate concern, however, is the increase in the volumes of the nuclei, and the fact that after invagination they "lie further apart than they did in the plate."

Morgan proved that there is no increase in the number of entoderm cells during invagination. The only factor which can be concerned in separating the nuclei is an increase in the volume of the extra-nuclear material. If such increase were the result of water absorption one would expect the increase in the size of the nuclei which was observed.

While this reasoning may be valid, nevertheless it is desirable to know whether nuclear size is a reliable index of the relative amount of water which the cell contains. That this actually is the case is indicated by certain measurements which I have

made on the unfertilized ova of *Asterias forbesii*.<sup>10</sup> These eggs constitute a very favorable material for the solution of this problem, not only on account of their size and shape, but also because their nuclei (germinal vesicles) are easy to see in the living cells and like these are spheres. In table 5 are given the diameters of eggs and nuclei which were first measured in normal sea-water, and afterwards in hypotonic.

From the figures in table 5 it is at once apparent that the cells absorb water in the hypotonic solution, and moreover that when they increase in volume, their nuclei do so likewise. It follows

TABLE 5

*Showing relative diameters of Asterias ova and their nuclei. The hypotonic sea-water was made by diluting six volumes of the normal sea-water, with four volumes of distilled water. The units of measurement are the same in all cases, but the exact absolute value cannot be given at this time. With greater as well as with smaller dilutions measurable changes in the same sense can be detected*

		NORMAL SEA-WATER	HYPOTONIC SEA-WATER
		units	units
Lot I	47 eggs.....	152.0	188.0
	47 nuclei.....	0.7	0.8
Lot II	49 eggs.....	154.0	170.0
	49 nuclei.....	0.7	0.8

from this that the volume of the nucleus may be taken as an index of the relative amount of water held by the cell, and that the increase in the size of the nuclei noted by Morgan during gastrulation, indicates an increase in the water content of the gastral plate. In this tissue, therefore, we have differential growth just as in the nervous system, and instead of being the outcome of the synthetic processes involved in growth by cell-multiplication, the increase in bulk is here also the result of water absorption.

The question as to the rôle of this absorption in the process of folding remains to be answered. As far as invaginate gastrulation is concerned, so long as the entoderm cells continue to have

<sup>10</sup> For an account of the methods employed see, Glaser, The change in volume of *Arbacia* and *Asterias* eggs at fertilization. Biol. Bull., vol. 26, p. 84.



the shape they possess in the blastula, an increase in volume could result only in stretching the ectoderm, and bending the gastral plate outward. If the increase in volume were to continue, the blastula would flatten, and finally the ectoderm, unable to stretch further would tear away from the entoderm. In the nervous system the situation would be exactly the same. The differential growth of neural and gastral plates alone therefore could never convert these structures into tubes, a conclusion reinforced by Roux's isolation experiments, for when a neural plate, completely isolated from other tissues folds itself, differential growth is certainly excluded. What significance then are we to attach to the water absorption, and how are we to explain the folding?

Rhumbler (*loc. cit.*) has pointed the way to an answer. The cells of the gastral and neural plates change in shape during invagination and folding. Since both processes are autonomous, an explanation would be found if we could show how the cells of themselves could change in form so that no other arrangement except that of the folded or invaginated state is possible. The factor possessing the necessary requirements, is, according to Rhumbler, differential surface tension.

Three cases are considered, as follows:

*Case A.* In the spherical blastula, all the cells are wedges whose smaller ends point toward the center. The outer poles of the entoderm cells are bathed by an external medium of relatively constant composition; the inner poles on the other hand project into the blastocoelic liquid whose composition is not only different, but no doubt changes during the course of development. For instance, the concentration of  $\text{CO}_2$  alone must be greater in this liquid than in the external medium which not only has a far greater volume, but into which ciliary convection currents are constantly driving the  $\text{CO}_2$  from the surface of the larva. Now the surface tension of a cell depends upon the nature of the surface and this is determined equally by the characters of two media which it separates. Rhumbler imagines that in the gastrula we have conditions under which the tension of the inner surfaces of the entoderm cells may well be different from that of the outer, and if it were less, then the outer surfaces

with their higher tension would, like elastic bags, squeeze the cell-contents inward. The inner surface under the circumstances would give way until an equilibrium resulted in which the entodermal wedges would be reversed. Theoretically such reversal is all that is needed to insure invagination or folding.

But why does this not apply equally well to the other cells of the blastula? Why do the entoderm cells alone invaginate? For the sake of simplicity, Rhumbler (*loc. cit.*, p. 448) considers first the mechanical conditions which may be supposed to obtain if the surfaces of the ento- and ectoderm cells are identical in physico-chemical composition. From the greater size of the entodermal elements it follows that their surface tension is less than that of the smaller ectoderm cells.<sup>11</sup>

If now the blastocoelic fluid lowers surface tension, it would bring about, per unit area, an equal lowering in all the cells, but in the entoderm, because of its absolutely greater surface, the absolute lowering would exceed that in the ectoderm.<sup>12</sup>

<sup>11</sup> The phenomenon of exo-gastrulation in lithium larvae (Herbst, *Arch. f. Entwicklungsmech.*, Bd. 2, p. 455, referred to by Gudernatsch, *loc. cit.*, p. 417) harmonize with the opinions of Rhumbler as expressed here, for in these embryos all the blastomeres are swollen and vacuolated, but just before exo-gastrulation the entoderm cells decrease in volume and become actually smaller than the ectoderm. This experimental fact harmonizes equally well with the somewhat freer interpretation which I shall present later on. In this connection it is interesting to recall the 'exo-neurula' of the frog, also produced by the use of lithium (Hertwig, Morgan). Gudernatsch (p. 423) also refers to Driesch's observation that gastrulation occurs in *Sphaerechinus blastulae* (*Mitteil. Zool. Stat. Neapel*, Bd. 11, p. 221), no matter whether they are derived from the micromeres or macromeres of the early cleavages. This fact does not preclude minute differences in the size of the cells in these 'partial' larvae, nor necessarily even differences in their chemical composition.

<sup>12</sup> The passage in Rhumbler (p. 449) in which this matter is discussed, reads as follows: "Die Entodermzellen sind aber grösser und haben desshalb auch eine 'absolute' grössere Oberfläche, so dass bei ihnen die Spannungsniedrigung wohlgeordnet 'pro-Einzelzelle' viel erheblicher ausfallen muss als bei den einzelnen Ectodermzellen, zumal die kleineren Ectodermzellen (weil sie von Haus aus eine grössere Oberflächenspannung pro Flächeneinheit besitzen), um sich zur Erzielung des gleichen Effektes nicht die gleiche, sondern eine entsprechend beträchtlichere Herabminderung der Oberflächenspannung pro Flächeneinheit verlangen würden, während ihnen doch nur eine gleiche geliefert wird." Gudernatsch (*loc. cit.*, p. 419) writes: "Rhumbler however believes that chemotaxis alone is the inducing factor of gastrulation."

Under these circumstances the entodermal cells would move into the blastocoel before the ectoderm, and the rise in internal pressure owing to the incompressibility of the blastocoelic fluid would render impossible any immigration or invagination on the part of any other cells.

*Case B.* The conditions of Case A are needlessly difficult, and were assumed simply to show that invagination is conceivable from this standpoint even under the most adverse circumstances. But the physico-chemical constitution of the surfaces of neither the gastral nor neural plate cells can be identical with that of the neighboring ectoderm for they do not enclose identical chemical systems. This is indicated not only by the localization of yolk and 'organ-forming' substances in the entoderm, and by the well-known cytological specificity of the early nerve cells, but especially by the specific water-holding capacity of the embryonic nervous system,<sup>12</sup> a specificity, identical with that of the adult system, and conceivable only as the outcome of a specific physical-chemical organization.

Rhumbler, for similar reasons, considers as a second possibility, "*dass die Zelloberflächen der sich einstülpenden Zellen so stark anders beschaffen wären als diejenigen der sich nicht einstülpenden Ectodermzellen*" (loc. cit., p. 450), that only the former react toward the blastocoelic fluid in the manner necessary to insure invagination.

*Case C.* Finally Rhumbler suggests that the greater size of the entoderm cells and their greater readiness to react appropriately to the influence of the blastocoelic fluid, might coöperate, and together bring about the decrease in surface tension required for the change in shape.

Although Rhumbler seems to me to place the emphasis where it belongs, certain qualifications appear to be either necessary or pertinent. In the first place, while the incompressibility of the blastocoelic liquid follows necessarily from the physical properties of fluids in general, it cannot be employed to explain

<sup>12</sup> See Glaser, The water-content of the embryonic nervous system. *Science*, vol. 39, p. 730.



in Case A and subsequent cases, why the ectoderm cells fail to invaginate, for if the fluid remained within the blastocoel, its incompressibility would of course render the invagination of the entoderm cells equally impossible.

In the second place, the explanatory value of 'surface tension' does not seem to me acceptable without certain reservations. That it may be the important factor, cannot be denied, neither can its importance be considered as demonstrated. Surface tension, together with the Gibbs-Thompson principle, gives an adequate physical explanation for the concentration of certain substances in the surface of the cell, but inasmuch as the substances so concentrated undergo changes in aggregation resulting in the formation of solid films, the application of the laws of surface tension meets with some difficulties. As long as we have no clear conception of the order of magnitude of the 'surface tension' of cells, and moreover lack really adequate methods of measurement, it seems rather questionable to lay too much emphasis on this particular factor. As Loeb<sup>14</sup> has pointed out, even in the relatively simple cases of amoeboid movement, mere changes in surface tension hardly seem adequate, for

If it is true that the *Amoeba* is covered with a solid film, one condition for the formation of a pseudopodium must be a local liquefaction of protoplasm. In consequence of such liquefaction new protoplasm must flow out, which subsequently will form a new solid film at its surface. This may again be liquefied, and a new streaming may occur, etc. Such liquefactions can be caused by lack of oxygen . . . . .; but they may also be caused by other chemical changes. I am inclined to believe that phenomena of liquefaction play at least some rôle in the processes of protoplasmic motion.

If such liquefactions occur in the neural and gastral plates, they, rather than alterations in surface tension, might be the important factors.

Until the possibilities have been experimentally limited or defined, it seems unwise to specify too carefully which particular surface effect, or combination of surface effects, is responsible for the change in shape undergone by the cells in the folding plates.

<sup>14</sup> Jacques Loeb, *The dynamics of living matter*, p. 57.

Nevertheless, even without this specificity, desirable as it would be, it is possible to apply Rhumbler's general thesis to the folding of the neural plate.

Let  $ABCD$  (fig. 3) represent a cell in the neural plate. Along the two sides  $AD$  and  $BC$  the cell is bounded by others like itself. The side  $AB$  limits the system toward the external world, the side  $DC$  toward the internal, but nevertheless extra-neural, environment. The shape and position of the cell is the expression of a state of equilibrium which depends not only on the nature of the physical-chemical system,  $ABCD$ , but equally upon conditions outside.

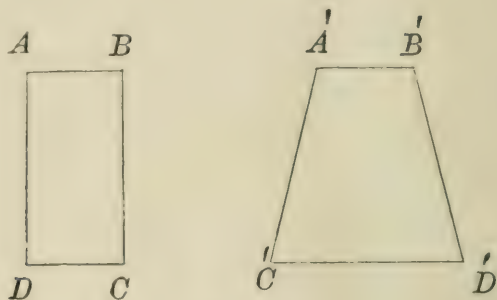


Figure 3

In the neural tube the shape of this cell is represented by  $A'B'C'D'$ , and this also is the expression of an equilibrium dependent on internal and external conditions. Obviously, however, since the shape and perhaps also the position of the cell differ from those it originally had, the new equilibrium is not indential with the old. Now the chances of a disturbance of equilibrium along the lines  $DA$  and  $CB$ , are not very great for the cell is bounded on these sides by chemical systems like itself. Along the line  $AB$  also the chances of disturbance are small, because here the cell abuts upon an external environment whose constancy is relatively high. Along the line  $DC$ , however, the cell is subjected to influences due to what is going on in the rest of the embryo, and as important changes are constantly occurring within every developing organism, it is certainly not

unreasonable to imagine that the internal environment, compared with the external, is relatively unstable. From the morphological standpoint, this is only another way of saying that development is taking place, from the physiological it is almost self-evident, and with reference to one chemical factor, at least, I know it to be true, for whereas *the frog's egg is neutral in its reaction to litmus, the contents of the young larvae, not yet hatched, are distinctly acid.*

Whether the transition from neutrality to acidity, or some other chemical change, is important, certainly the relative inconstancy of the internal extra-neural environment is no assumption. Rhumbler attributes a similar instability to the blastocoelic fluid, and imagines that when certain substances have reached a certain concentration, a lowering of the surface tension in the entodermal plate will result. Correspondingly, if such a lowering should occur in the neural plate, the side  $AB$  would lengthen, the cell would assume the shape  $A'B'C'D'$ , and the observed translocation of intra-cellular contents, and necessarily also the observed folding, would be accounted for.

For reasons already given, I prefer to assume, instead of a lowering of the surface tension, simply 'a surface effect.' This does not exclude the factor emphasized by Rhumbler, but leaves room for such other possibilities as liquefaction, 'etching' and changes in permeability. Any of these, singly or in combination might result in a weakening of the internal surface, and though the effect of such weakening would be identical with that brought about by a lowering of the surface tension the actual mechanism might nevertheless be quite different. But what evidence have we for this 'surface effect'?

A surface effect necessarily involves a change in permeability, and a change in permeability may be followed either by a gain or loss of water.<sup>15</sup>

Since now the nervous system demonstrably, and the entoderm, probably, increase in volume during folding, and since

<sup>15</sup> Glaser, On inducing development in the sea-urchin (*Arbacia punctulata*) together with considerations on the initiatory effect of fertilization. Science vol. 38, p. 446.



this increase is the outcome, not of cell-multiplication, but of water absorption, a surface effect, involving a change in permeability is practically certain. Since the sense of this change, for water at least is positive, it seems likely that the affected surface would be mechanically weakened by it.<sup>16</sup> With the initiatory changes in folding accounted for on this basis, the possibility that the absorption of water has after all a 'formative' influence, once more arises, for even if an increase in volume cannot of itself induce folding, it might accelerate or retard the process when initiated by other forces.

A colloidal rod or tube which bends in boiling water can easily be shown not to have a uniform dry substance, and a board may warp through differential water absorption. Since the water must enter the neural plate via the affected surface, a differential localization would greatly facilitate folding.<sup>17</sup>

I have tried many experiments for the purpose of detecting such differences in the intra-cellular concentration of water, but have not succeeded in finding any evidence of differential distribution. Indeed on physical-chemical grounds, such an arrangement of the water does not appear very likely. It is of course still less likely to be distributed so as to oppose the folding. Most probably the distribution is what one might expect, an arrangement, which indeed affects the size of the cells, but has no other formative, or morphogenetic significance at this particular stage of development.

<sup>16</sup> See Höber, *Physikalische Chemie der Zelle und der Gewebe*, 3rd ed.

<sup>17</sup> In the case of the gastral plate, the increase in size must be due to the absorption of water from the blastocoel. As a matter of fact the blastocoelic fluid decreases in amount, and this decrease is absolutely essential for invagination, not because the absorbed water is differentially distributed in the entoderm cells, or because its removal from the cavity produces a suction, but because in this way an insurmountable obstacle, an incompressible liquid, is removed. The following experiment is not without interest in this connection. Normal free-swimming *Arbacia* blastulae placed in sea water diluted with an equal quantity of distilled, absorb a great amount of water of which part enters the blastocoel and part remains in the cells themselves. The development of such inflated blastulae is arrested, but if returned at the end of 24 hours to normal sea water, they instantly regain their former size, and development once more proceeds normally.

## VIII. SUMMARY

We may summarize the foregoing considerations as follows:

1. The folding process by which the neural plate becomes converted into a neural tube, does not depend on coercive pressure from without, and in this sense is autonomous (Roux).

2. The cells of the neural plate, actively engaged in folding undergo the change in shape emphasized by Rhumbler in the invaginating gastral plate.

3. During the period of involution in *Cryptobranchus* cell-multiplication appears to proceed at a negligible rate, a conclusion practically identical with the inference drawn by Morgan from a study of gastrulation in *Sphaerechinus*.

4. If the folding nervous system be divided into an inner and outer zone, an outward migration of nuclei during involution is demonstrable. Translocation of intra-cellular contents also occurs in invaginate gastrulation (Rhumbler).

5. During involution no doubt the outer zones increase at the expense of the inner, but the exact extent of this is difficult to determine since the inner zones also increase.

6. This 'growth' of the nervous system is not the result of the synthetic processes ordinarily associated with cell-multiplication, but is the outcome of water absorption.

7. The folded nervous system contains 80 per cent of water and 20 per cent of dry substance; the entire embryo, on the other hand, has only 58 per cent of water and 42 per cent of dry substance. For the isolated yolk-sac of the frog's embryo the corresponding figures are 55 per cent and 45 per cent respectively. During involution, therefore, differential water absorption takes place in the nervous system.

8. Such differential absorption can also be inferred for the entoderm from Morgan's observations on the gastral nuclei of *Sphaerechinus*, for these increase in volume and lie further apart after gastrulation than before. These changes would be expected if the gastral plate absorbs water at this time.

9. As shown by the behavior of *Asterias* ova in normal and hypotonic sea water, nuclear size may be used as an index of the relative water content of the cell.

10. The 'morphogenetic' or 'formative' effect of the water absorption is in all probability zero. The size of the cells is of course increased, but such increase can only affect the process of involution if the absorbed water is differentially distributed in the cell. For this there is no evidence, and little probability. The real significance of the water absorption seems to lie in the fact that it is a symptom of a surface effect which involves apparently a change in the permeability of the neural plate cells.

11. The surface affected is more likely to be the one bounded by the extra-neural, intra-embryonic environment than any other.

12. The contents of the frog's egg are neutral to litmus; those of the larva not yet hatched, acid.

13. On the basis of these facts and certain other considerations, it is proposed to modify Rhumbler's theory of autonomous folding by substituting 'surface' effect for 'surface tension.' This does not exclude surface tension from the list of possible factors, but leaves room for others such as liquefaction of the surface, 'etching,' and changes in permeability, all of which are possible in solid films.

14. The surface effect indicated by the absorption of water during folding, may very possibly result in a mechanical weakening from which the involution of the neural, and the invagination of the gastral plates follow, not only automatically, but with the demonstrated autonomy.

#### POSTSCRIPT

After this manuscript had been completed, I discovered the very recent and important contribution of Gurwitsch, "Der Vererbungsmechanismus der Form" (Arch. f. Entwicklungsmech., Bd. 39, p. 516).

This work falls naturally into two divisions, one theoretical, the other dealing with concrete observations. Inasmuch as the theoretical discussion involves the conceptions of 'Partialzweck' and the 'dynamisch präformirte Form' I must postpone, perhaps indefinitely, the attempt to enter these difficult regions.



With respect to the concrete results of Gurwitsch, it is to be noted that the distribution of the nuclei in the folded regions studied by him, is identical with the distribution I have found. However, there are also significant differences, particularly in connection with the rôle assigned to cell-multiplication and cell-migration, but these 'discrepancies' are not necessarily indicative of errors, instead they may be only the inevitable results of dealing with two quite different periods of development as well as with different materials. The neural plate of *Cryptobranchus* shows that folding can take place without cell-multiplication, and the conclusion that it does so, is in no wise affected by the frequent occurrence of mitoses in the corresponding stages of other forms. These constitute a less favorable material inasmuch as they do not present the simplest case. However, even these cases may prove to be instructive for the localization of the mitoses in the mammalian neural plate is such that cell-multiplication, if effective at all, would not facilitate, but oppose the process of involution.

I am inclined to hazard the guess even now, that the cell-migrations in the folds studied by Gurwitsch are effects rather than causes, but as I shall approach these stages from another angle, in a forthcoming paper, I shall reserve until that time a full discussion of the bearing of Gurwitsch's basic observations. In the meantime, in order to avoid misunderstanding and anticipate its attendant needless difficulties, I should like to impress on the reader as strongly as possible, that the mechanism of the process so carefully analysed by Gurwitsch, involves factors which are not concerned in the autonomous folding of the neural plate. In the present instance, the assumption that the mechanics of every folding process are identical with those of every other, would certainly lead to erroneous conclusions.

## BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

**DISSECTION METHODS AND GUIDES.** David G. Metheny, M.D., L.R.C.P., L.R.C.S. (Edin.), L.F.P.S. (Glas.), Associate in Anatomy and for sometime senior demonstrator in the Daniel Baugh Institute, the department of Anatomy and Biology, Jefferson Medical College, Philadelphia, 131 pages, illustrated, 1914, \$1.25 net. Philadelphia and London: W. B. Saunders Company.

"This book is intended to bridge the gap that exists between the descriptive text-book and the dissecting table. It is designed for use in conjunction with a text-book, but it is not to supplant it in any way. In order that it may be used in connection with any text-book or atlas, both the old and the new anatomical names have been given. If the instructions seem to be too minute, it should be remembered that the student's first effort may happen to be that very dissection; therefore nothing has been left to chance. Everything that a student could reasonably be expected to do in any well-equipped dissecting room has been carefully explained. Some of the dissections are original, and all of them have been carefully selected with a view to their being well within the capacity of the average student to perform." From the Introduction.

**ANATOMY OF THE HUMAN SKELETON.** J. Ernest Fraser, F.R.C.S., Eng. Lecturer on Anatomy in the Medical School of St. Mary's Hospital; formerly lecturer at King's College, London; and senior demonstrator at the Medical School of St. George's Hospital; examiner in Anatomy for the Conjoint Board of the Royal Colleges of Physicians and Surgeons, 274 pages including Index, 219 illustrations in black and color, 1914, \$6.50. Philadelphia: P. Blakiston's Son & Co.

"It is not necessary to lay emphasis on the importance of a knowledge of the skeleton as an integral part of the study of human anatomy, and, in the literature bearing upon the subject, we find masterly accounts of the constituent bones, which rank as classics in the education of the student. In this book I have ventured to wander in some degree from the well-trodden road and to lead the reader by other ways to the comprehension of his subject. My intention has been to induce him to think of the bones as they exist in the body rather than as they lie on the table before him, and to do this I have laid stress—because he must use the prepared specimens—on the meaning of small details and on the relations of the bone, and have relegated the pure description of the dry bone to a secondary place: in other words, each part of the skeleton has been used as a peg on which to hang a consideration of the neighbouring structures, in the hope that this may afford a new point of view to the reader and enable him to grasp the intimate connection between them." From the Preface.

**MORRIS'S HUMAN ANATOMY**, a complete systematic treatise by English and American Authors, Edited by C. M. Jackson, M.S., M.D., Professor and Director of the Department of Anatomy, University of Minnesota. Fifth edition, revised and largely rewritten, 1539 pages including Index, 1182 illustrations of which 358 are in colors, 1914, \$6.00. Philadelphia: P. Blakiston's Son & Co.

"One criticism upon most of the current text-books of human anatomy is that they are too extensive for the beginner. Much precious time is wasted by him in floundering through a mass of details which obscure the fundamental facts. And yet it is important to have these details conveniently accessible for both present and future reference. To meet this difficulty, the attempt is made in this edition to discriminate systematically in the use of sizes of type. The larger type is used for the more fundamental facts, which should be mastered first, and the smaller type for details. While it has been found difficult to apply this principle uniformly through the various sections, it is hoped that the plan, even though but imperfectly realized, will prove useful to the beginner." From the Editor's Preface.

The contributors to this Fifth Edition are: Charles R. Bardeen, Eliot R. Clark, Irving Hardesty, C. M. Jackson, F. W. Jones, Abram T. Kerr, J. Playfair McMurrich, John Morley, H. D. Senior, R. J. Terry, Peter Thompson, David Waterston.













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